ENVIRONMENTAL CLUTTER ELICITS BEHAVIORAL ADAPTATIONS DURING NATURAL BEHAVIORS IN ECHOLOCATING BATS

by
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ABSTRACT

The natural environment is filled with clutter that creates challenges for animals participating in routine tasks like orientation, foraging, and communication. For echolocating bats that primarily rely on an active sensory system, the effects of acoustic and physical clutter become more prominent with the potential to completely degrade the individuals' ability effectively navigate their environment or engage in prey capture. From sonar jamming moths and competitive conspecifics to rapid prey pursuit in dense forests, bats must quickly adapt their sensory-guided flight behaviors in real-time to remain effective aerial predators. To explore the sources of acoustic clutter and their effects on natural bat behaviors, a literature review is presented on the historical and current perspectives on sonar jamming and the underlying mechanisms of the jamming avoidance response. This is followed by experimental evidence of bats making use of a jamming avoidance response when presented with playback of heterospecific bat calls thought to decrease foraging efficacy. Bats were found to significantly alter their individual echolocation call features in a manner that is thought to improve the signal-to-noise ratio, which would aid in the increased detection of their own echoes. The remaining chapters explore how bats might make use of multisensory cues when environmental conditions are unfavorable for echolocation alone and how bats adjust their flight strategies when navigating in a novel environment filled with physical clutter. These chapters respectively report that multimodal cues comprised of visual and acoustic information lead to enhancement of responses during an obstacle avoidance task and that significant changes in flight kinematics can be observed in cluttered vs. open environments.

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DEDICATION

To my wife. You're simply the best.

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Chapter I

Introduction

Bats (Chiroptera) are a fascinating and diverse order of mammals that display a broad array of morphological and behavioral adaptations that have allowed them to dominate the night skies as the only mammals capable of sustained flight. Their specialized sensory and motor systems have given them unparalleled ability to exploit almost every terrestrial habitat and food type. Most species of bats use echolocation, an active sensory system, to probe their environment by emitting high-frequency acoustic signals and extracting relevant information about their surroundings from the echoes returned by nearby objects (Griffin 1958). This is an extremely demanding task for the auditory system in that bats must rapidly transform information carried by soundwaves into useful perceptual images to support decision-making in complex environments, often while moving at high speeds in three dimensions.

Cave-dwelling species, such as *Tadarida brasiliensis*, must coordinate large nightly group emergences consisting of sometimes millions of individuals during which they need to avoid obstacles (and each other) moving at average speeds of 14 m s^{-1} (McCracken *et al.* 2016) and somehow discern relevant acoustic stimuli amid echolocation calls, returning echoes, and social calls. The majority of the Microchiroptera[i], especially in temperate regions, prey on insects

[i] Microchiroptera and Megachiroptera are the two traditional suborders used to differentiate the former as the echolocating bats and the latter as mostly non-echolocating fruit bats in the family Pteropodidae that rely primarily

(Fenton and Simmons 2015) and many of these species do so by catching and consuming them in flight. This can lead to bats experiencing auditory masking generated by their own sonar vocalizations (Schnitzler *et al*. 2003), in which the echolocation call interferes with the detection of target echoes. Species that glean insects from surfaces, as well as those who pursue and eat insects on the wing in dense foliage, are subjected to backward masking from echoes returning from environmental clutter. How do bats perform such complex tasks of orientation, obstacle avoidance, and prey capture in spaces that are filled with physical and acoustic elements that create challenges in flight and active sensing?

Adverse Acoustic Conditions

A critical, but sometimes overlooked aspect of conducting research on a single sensory system, is the inherent limitations associated with that modality. Experiments are designed to answer questions that accentuate the strengths of the modality of interest, often presenting stimuli under conditions optimal for signal detection and with little regard to how other systems may contribute to natural behaviors in biologically relevant scenarios. However, it is important to realize that animals, including humans, rarely, if ever, operate solely based on the information coming in through a single modality. This is not to say that a dominant modality, at least during some behaviors, is not present. Humans and many other diurnal animals are largely regarded to be visually dominant (Spence *et al*. 2012), while many nocturnal and crepuscular animals evolved

on vision (Prothero 2017). More recently, taxonomic studies have supported dividing the order into Yinpterochiroptera, to include the megabats along with the microbat superfamily Rhinolophoidea, and Yangochiroptera, the remaining microbats (Wilson and Mittermeier 2019). For the purpose of this dissertation, I use the traditional convention to differentiate echolocating from non-echolocating bats, without making any claims to the assumed evolutionary relationships. "Bats" without modifiers refers to echolocating bats.

to accommodate vision in low-light and/or began to rely on other faculties such as somatosensation or olfaction.

Among animals in the latter group, bats evolved to rely on their hearing more heavily than any other sense. Encoded in the returning echoes of sonar emissions from the nose or mouth is information about the properties of objects in the environment, including distance, size, shape, orientation, texture, and motion parameters (Simmons 1973; Kick 1982; Roverud and Grinnell 1985; Moss and Surlykke, 2010). Additionally, echolocation is an adaptive system in that bats can modify sonar call parameters[ii]. This adjustment is in response to feedback gathered from the environment and is refined to meet the requirements of specific tasks through the alteration of spectral and temporal features.

The two primary limitations of using echolocation are 1) that it is subject to acoustic interference, or "sonar jamming," and 2) that the intrinsic physical constraints of the signals drastically reduce the kind of scenarios in which echolocation is useful (i.e. it is not useful for long distances or large objects). However, there are multiple ways bats can overcome these limitations. Bats faced with sources of acoustic interference from other bats, insects, abiotic factors, or anthropogenic noise may experience a disruption in their ability to successfully receive and process returning echoes. The adaptivity of sonar features lends itself to being able to change the spectrotemporal structure of the calls to overcome the effects of signal interference or jamming. When facing challenges imposed by the physical constraints of echolocation, bats may incorporate information from other sensory modalities to fill in the missing pieces. For example,

[ii] This plasticity in echolocation emissions is not without constraint, creating species-specific repertoires that are most suited for their individual ecological conditions (Schnitzler and Kalko 2001).

bats may use vision to identify large, distant objects in the environment which has been noted in bats using landmarks to guide their commutes between roosts and foraging grounds or during migrations (Griffin 1970).

The first three chapters explicate how bats utilize auditory and visual adaptations to guide natural behaviors in potentially disadvantageous acoustic conditions. I address this first by presenting a comprehensive review of the relevant literature surrounding sonar jamming and the jamming avoidance responses in Microchiroptera. This extensive analysis of prior experiments set the basis for Chapter 2 in which I describe how bats might overcome acoustic interference when foraging. This study (Jones *et al*. 2018) demonstrated that *Eptesicus fuscus* presented with 'jamming signals' previously documented to result in missed prey items (Corcoran and Conner 2014) did not show a decrease in prey capture successes when the experiment was repeated. Bats were trained to capture tethered moths in a large flight cage and were randomly presented with either silence or one of several natural or synthetic bat calls played through a speaker near the prey items. However, playback of the stimuli did result in changes in the bats' echolocation strategies. Though it can be difficult to interpret these changes in isolation, holistically it is most likely that changes in the bats' sonar signal design increases signal-to-noise ratio, which would allow bats to pick out the echoes carrying the most relevant information out of the surrounding din. This generally falls under the umbrella term of "jamming avoidance response," which includes many changes a bat, or other animal using active sensing, can utilize to avoid the effects of disruptive environmental signals.

Because bats encounter situations in which they are subject to acoustic clutter and interference or when acoustic feedback is not reliable, it is plausible that bats might rely on other sensory modalities, when available, to compensate. There is a small collection of literature

concerning information on the use of vision by bats so Chapter 3 is devoted to the execution of preliminary experiments that revealed how bats might integrate information from the visual system to guide their behaviors during an obstacle avoidance task under conditions when echolocation alone is insufficient. Bats were trained to fly into a restricted area that was either open or obstructed. Obstructions were created to either return an echo, be visible, or to be visible and return an echo which forced bats to differentially rely on each sensory modality during task completion. Results suggest that some behavioral responses can be enhanced by the presence of visual information in conjunction with acoustic information. These data, along with those published in recent years, illustrates the complementary nature of vision, alongside echolocation, under environmental conditions that support multimodal sensing (Boonman *et al*. 2013).

Adverse Physical Conditions

Bats are constrained in their perception of the world by the limitations of their sensory systems (echolocation, vision, olfaction, passive listening, somatosensation) and mechanically in their motor capacities. As such, flight performance in these animals should reflect a combination of biomechanical constraints and evolutionary adaptations that would yield solutions to the demands of powered flight in the form of behavioral modification and morphological tailoring. Where studies have documented distinct adaptations in bat echolocation patterns across ecological niches and linked this with physiological traits, such as nose leaf or ear characteristics (Arita 1990; Keeley *et al*. 2018), it should follow that ecological constraints would also result in similar relationships between wing morphology and flight performance. Thus, it is likely that bats possess species-specific strategies for compensating in adverse physical conditions, such as the presence of obstacles.

In the final chapter, I present a preliminary study on quantifying flight performance in a species that regularly orients and forages in the dense foliage of a tropical island habitat. *Hipposideros alongensis* flew in a tent at a field site in Vietnam in either an open condition, in which there were no obstacles, or in a cluttered condition, in which the bat had to navigate around obstacles. 3D trajectories of the flights were reconstructed and analyzed to yield the first data set on flight parameters for this species and show that significant changes in features such as velocity and turn rate can be observed when bats fly under different environmental conditions along with individual variation.

Summary

In this dissertation, I explore how adverse acoustic and physical environments challenge echolocation signal processing and impact flight performance, and document how bats adapt in real-time. It is important to study how bats are able to cope with such problems in contexts that are, or mimic as closely as possible, ecologically relevant scenarios. The experiments reported here use two of the most ecologically relevant tasks for the majority of bats: prey capture and obstacle avoidance. Collectively, these experiments show that to remain successful aerialists, bats require exceptional detection and localization skills, superior flight capabilities, as well as the ability to integrate multiple sources of stimulus input.

Chapter II

Perspectives on signal jamming and the jamming avoidance response

Chapter Notes

The background research for this chapter began early in my graduate career. I amassed dozens of research articles discussing the flexibility of biosonar in bats and the challenges they face when their environments are filled with various sources of acoustic interference. All of this combined with the relatively sparse quantitative studies on sonar jamming by moths and conspecifics led to the first drafts of this detailed review. Here, I try to address the concerns and controversy that have been documented in various articles, as well as those registered in person from colleagues, to provide a thorough account of jamming and the jamming avoidance response in bats. In addition to historical perspectives originating with the studies of weakly electric fishes, I tentatively explore the possible underlying neural mechanisms that may mediate bats' responses to signal jamming.

Abstract

To successfully navigate the world, every animal must deal with the challenge of extracting meaningful signals amid an environment filled with noise. For echolocating bats, this noise comes in the form of various types of acoustic interference, or sonar jamming, causing degraded performance of their active sensory system. To compensate, bats alter their highly flexible call repertoire in the form of jamming avoidance responses. Here, we discuss the history of jamming and jamming avoidance responses and detail the most common sources of acoustic interference. Additionally, we examine the various definitions of jamming avoidance responses and how changes in signal design can alter a bats' acoustic image.

I. Active sensory systems in fish and bats

Many animals possess active sensory systems that trigger sensory receptors with self-generated energy to navigate their environments and to forage for food. Using active sensing allows for precise control over critical parameters necessary for executing these tasks such as signal intensity and directionality. Organisms benefiting from such sensory systems are often found operating in environments where other forms of sensory input, such as vision, are of little use, such as deep or murky water or in dim-light environments. The most common examples stem from electrolocation and echolocation, two active sensing systems using very different signals to accomplish similar goals.

The most basic principles of active sensory systems such as biosonar is to emit some signal, whether it is acoustic or electric, and to then compare it to the returning distortions of that signal. The resulting differences in parameters, such as arrival time and spectral composition, are used to estimate target properties such as range, size, and velocity. These naturally occurring systems are complex and sophisticated and have served as a model for human applications of similar technologies (i.e. sonar, radar, and lidar) in various capacities, from war to topographic mapping.

The most well-studied active sensing systems reside in the weakly electric fishes, in the form of electrolocation, and Microchiropteran bats, in the form of echolocation[iii]. It is from these

[iii] Odontocetes also utilize echolocation through different anatomical mechanisms for foraging and navigation through aquatic environments with low visibility. The parameters of echolocation emissions vary between the toothed whales and bats, partially due to the properties of the media through which the sound travels (i.e. water vs. air). A full review of the major differences in the two echolocation systems is documented by Au (1997). There is far less literature studying the echolocation of odontocetes than bats and even less documenting their responses to interference. A recent study by Kloepper and Branstetter (2019) suggests that, while dolphin echolocation may not be as flexible as bat echolocation overall, they still demonstrate spectral and temporal changes in their calls when presented with acoustic interference while completing a task.

two systems that we extract the definitions of signal jamming and derive similar, but distinct, definitions of jamming avoidance responses (JARs) in biological systems.

I.I Electrolocation

Electroreceptors are not uncommon in vertebrates (Bullock *et al.* 1993). Many fishes (Hopkins 1976; Kajiura and Holland 2002), amphibians (Himstedt *et al.* 1982; Fritzsch and Wahnschaffe 1983), and mammals (Scheich *et al.* 1986; Czech-Damal *et al.* 2011) have demonstrated the ability to sense electric currents when navigating and/or finding prey. The ability to emit electric signals, as well as receive them, is reserved for the electric fishes, which typically fall into the strongly electric fishes (SEF) and weakly electric fishes (WEF). These fishes possess electric organs derived from muscle or nerve tissue (Bennett 1971). Electric organs are composed of stacked electrocytes that function similarly to a series of batteries and generate voltage gradients. SEF produce a much higher voltage output (10-600 volts) that is used in defense and to stun prey items (Heiligenberg 2012). WEF, use their low voltage (<1 volt) signals for object detection (von der Emde *et al.* 1998; von der Emde 1999) and interspecific communication (Hagedorn and Heiligenberg 1985).

Weakly electric fishes are capable of using their electric organ discharges (EODs) to generate an electric signal and then sensing the resulting distortions in that signal due to nearby objects via electric receptors. Electric receptors are located in the epidermis and sense changes in current flow that create an electric image across the skin and are the result of varying properties of nearby objects, such as conductivity, size, shape, and distance.

Although some species demonstrate detectable individual differences in their EODs (McGregor and Westby 1992), often when fish are faced with the interfering electric signals of a

nearby conspecific, they exhibit a traditional JAR in which they actively alter their electric emissions to minimize interference. The type of JAR exhibited is dependent on the type of electric emission generated. Fishes that emit pulse-like signals will change the interpulse interval to avoid temporal coincidences while those that emit wave-like signals alter change their discharge frequency to avoid spectral overlap (Fig. 2.1). It was from these studies in electric fish that our first predictions about JARs in echolocating bats arose.

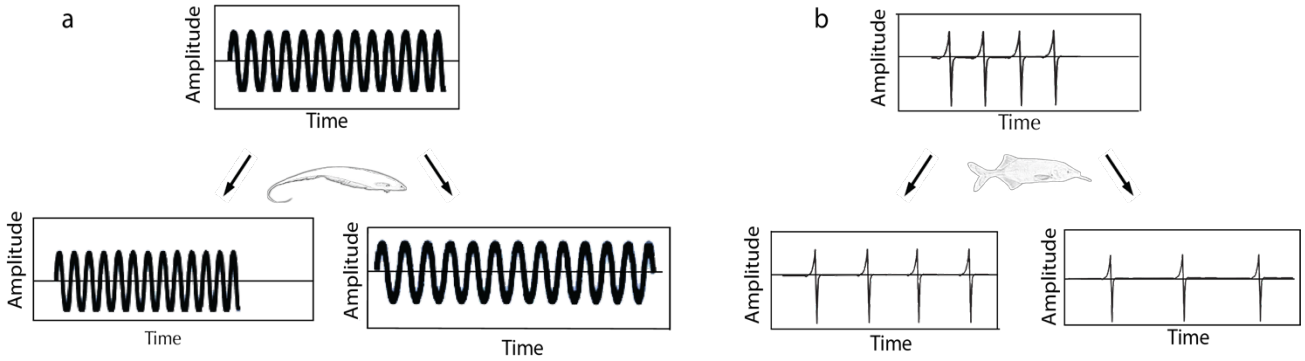

Figure 2.1. Examples of jamming avoidance responses in a) wave-type and b) pulse-type weakly electric fish species. Two individuals in close proximity emitting the either similar frequencies or temporal patterning of EODs (top graphs) will both alter their emissions to increase signal disparity.

I.II Echolocation

Bats (suborder Microchiroptera) often forage in conditions where their visual system is ineffective and thus use active biosonar emissions, or echolocation for hunting and general navigation. Bats echolocate by making rapid, high-frequency calls produced by the larynx and emitted through the nose or mouth, often at high intensities and can then extract critical information about their surroundings from the returning echoes off nearby objects or potential prey items (Popper and Fay 2012). Echolocation allows bats to orient in complex environments, identify and navigate to roost sites, forage for food – many species pursue small insects and catch

them on the wing. This variety of applications for echolocation is possible due to the impressive flexibility of the emitted signals. The echoes a bat receives are largely dependent on the characteristics of their emitted calls and they will change both individual calls and patterns of multiple calls. They are capable of altering numerous parameters (Fig. 2.2) including call duration, bandwidth, intensity (reviewed in Grinnell 1995) and do so in response to the surrounding environmental conditions (Moss and Surlykke 2001; Hiryu *et al*. 2010).

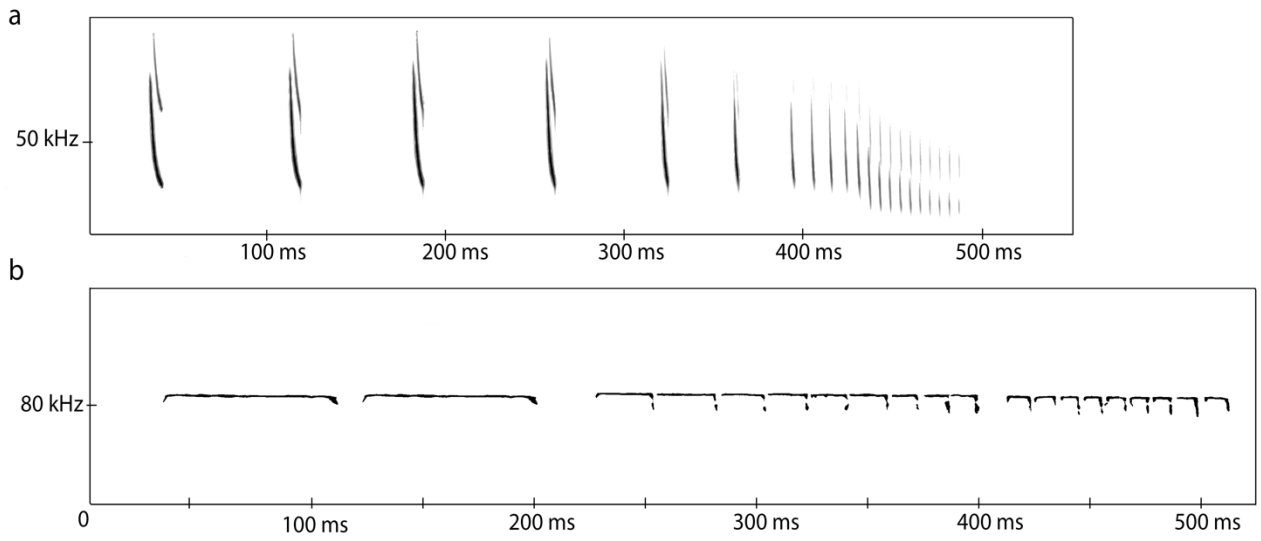

Figure 2.2. Prey pursuit sequences for an FM and CF-FM bat. Prey pursuit sequence for a) the FM bat, *Myotis lucifugus*, and b) the CF-FM bat *Rhinolophus ferrumequinum*; adapted from Clare *et al*. (2014) and Vogler and Neuweiler (1983), respectively. In both species, rapid dynamic changes are observable, most notably in the temporal patterning of decreasing pulse intervals as the bat closes in on its target. In FM bats, we also often observe frequency changes, such as decreased bandwidth as the bat executes its terminal buzz.

Bats can produce sounds as low as 11 kHz (Fullard and Dawson 1997) and as high as 212 kHz (Fenton and Bell 1981) depending on the species. For insectivorous bats, these higher frequencies are required if the returning echoes are to be strong enough to detect insects (Houston and Boonman 2004) and typically use frequencies between 20 – 60 kHz (Fenton *et al.* 1998). However, because high frequencies are subject to greater atmospheric attenuation, the range of echolocation is limited (~100 m). The intensity of bat echolocation calls are some of the highest recorded for vocalizations of any airborne animal (Jones and Holderied 2007a) and have been documented as high as 133 dB SPL at 10 cm from the bat (Holderied and von Helversen 2003). These calls have the potential to be so loud that bats employ a mechanism to avoid self-deafening by contracting the muscles in the middle ear (Jones 2005).

Bat echolocation is broadly grouped into two categories: those that use constant frequency (CF) and frequency-modulated (FM) calls. CF-FM bats emit long duration sonar emissions that do not vary in frequency, but often include a short FM sweep at the end. These bats also exploit Doppler shift compensation in which they alter their own emitted frequencies and use the returning information to determine the speed and direction of the prey item (Busnel, René-Guy and Fish 1980; Trappe and Schnitzler 1982). Rather than a single frequency, FM bats "sweep" through approximately an octave and often contain multiple harmonics that act to increase overall bandwidth. These calls are generally several milliseconds long and are emitted at intervals of ~50 ms or more during the search phase (i.e. when bats are cruising in a particular area prior to prey detection). Once a bat has detected a potential prey item, its calls are emitted more rapidly (12-40 ms intervals) until the terminal buzz (<12 ms intervals) right before the bat captures its prey. FM calls are often employed by species hunting in cluttered environments (Simmons 1979; Schnitzler *et al.* 2003) and is well-suited to target localization. FM calls have

better range resolution due to the comparison of pulse-echo time delays and the size of a target can be perceived from the intensity of the echoes (Simmons and Vernon 1971) while shape can be determined by the echo spectrum (Simmons *et al.* 1974).

Bats exhibit anatomical and neural adaptations related to echolocation. Almost all echolocating bats produce sounds via the larynx, which contain superfast muscles (Elemans *et al.* 2011) needed for rapid sound emissions at the millisecond timescale. In the inner ear, bats possess a large cochlea (Pye 1966) with a stiffer and relatively longer basilar membrane (Küçük and Abe 1992; Kössel and Vater 1995), increasing sensitivity to higher frequencies. Bats also possess the shortest outer hair cells and this is thought to be an adaptation to their high-frequency hearing range (Adams and Pedersen 2000) and these are present throughout the cochlea rather than being restricted to the basal end (Kuhn and Vater 1995). Externally, the pinnae are capable of rapid shape deformations to change spectral features of sounds entering the ear (Gao *et al.* 2011) and can also act as an amplifier. The auditory neurons of the brain are sharply tuned and can encode very small differences in frequency, intensity, timing and recover rapidly from stimulation which is essential for short pulse-echo delays (Simmons and Young 2010).

Bats are the only mammals that can rely almost exclusively on echolocation and adaptations of the mammalian auditory system make this possible. Over the last 60 million years (Teeling 2005; Simmons *et al.* 2008), they have evolved a highly sophisticated and flexible active sensory system to enable them to occupy a nocturnal aerial niche and this makes them not only extremely interesting, as we humans find it difficult to function without vision, but also an excellent model system for studying acoustic behavior and neural processing.

II. Sources of acoustic interference

Animals relying on echolocation are subject to a myriad of sources of acoustic interference, or sonar jamming, and the previously described echolocation flexibility is important for handling sounds that impede normal echolocation. Based on the historical studies of both WEF and bat active sensory systems, we can define signal interference, or "jamming", as the result of any signal within the same sensory domain that interferes with the detection, discrimination, or localization of a target signal. This should be behaviorally indicated by either a decrease in task performance and/or the modification of subsequent signal emissions.

For the purpose of this paper, acoustic interference is classified as either passive or active. Passive interference, or background noise, is that which is not necessarily intended to interfere with echolocation. Active interference sounds, or jamming signals, are those that are produced to with the purpose of interfering with echolocation. These sounds are most often generated by prey items as a defense or by competing conspecifics.

II.I. Passive acoustic interference: background noises of the environment

There are several sources of ambient noise in the environment such as insects, running water, and anthropogenic sounds. The sounds of nearby conspecific and heterospecific bats also have the potential to interfere with echolocation.

Bats have a propensity to forage in close proximity to one another, with individuals often "eavesdropping" on others to find areas with abundant food (Cvikel *et al.* 2015), but their ability to do so effectively is density-dependent. In small groups, bats demonstrate that they are not limited in their ability to navigate the environment and capture prey (J.A. Simmons 2005; Moss

et al. 2006) in the presence of others, but as groups grow larger, conspecific interference limits prey capture (Cvikel *et al*. 2015).

II.II. Active acoustic interference: sonar jamming in insects and bats

Sonar jamming in insects is predicated on two conditions. The first, hearing[iv], is a necessity to detect approaching bats. Unlike, chemical defenses, being able to hear a nearing predator provides an opportunity to enact some escape strategy prior to handling, which can still result in severe injury or death, if not consumption. Second, the organism must possess sound producing organs. Among insects, there are a variety of structures to produce sound, including stridulatory organs, files, the beating of wings against elytra, and our focus, the tymbal organ.

Hearing structures have evolved independently in several orders of insects and their functions range from intraspecies communication to predator detection (Hoy *et al*. 1989; Yager 1999). Hearing mediated by tympanal ears has evolved approximately 18 times in the insects (Strauß and Lakes-Harlan 2014) and the sensitivities of tympanal organs generally match the calls of echolocating bats (Fullard and Barclay 1980; Surlykke 1986; Fullard 1988; Yager 1999; ter Hofstede *et al*. 2013). Lepidoptera (moths and some nocturnal butterflies), Coleoptera (beetles), Orthoptera (crickets, grasshoppers, locusts), Dictyoptera (mantids), Neuroptera (green lacewings), and Diptera (flies) all hear bats and have evolved a variety of anti-predation strategies (Miller and Surlykke 2001). Acoustic forewarning is one of the primary drawbacks of foraging with echolocation and plays a critical role in the diffuse coevolution between insects and bats.

Once in possession of ears broadly tuned to the range of frequencies used by their

[iv] It is important to note that sound production itself is not dependent on the ability to hear as some hawkmoths possess sound-producing organs but lack an apparent hearing structure (Kawahara & Barber 2015).

echolocating predators, insect defenses improved. Some developed evasive maneuvers that could take them out of the path of an attacking bat (Roeder 1963; Miller and Surlykke 2001) while some were able to produce their own high-frequency sounds (Blest *et al*. 1963; Dunning 1968; Barber and Conner 2006; Corcoran *et al*. 2010). Sound production in insects is mediated by the buckling of ventro-laterally placed tymbal organs on the thorax (Tougaard *et al*. 2003). Each tymbal may have a row of minute corrugations or microtymbals, potentially affecting the number of successive ultrasonic 'clicks' produced (Fenton and Roeder 1974). The basalar muscle controls the contraction of the tymbals and the length of the contraction cycle determines the duration of the click cycle (Fenton and Roeder 1974).

In many tiger moths[v] (Erebidae: Arctiinae), the function of these clicks is hypothesized to be acoustic aposematism (Surlykke and Miller 1985; Hristov and Conner 2005a). Such a warning signal serves to inform bats that the moth is unprofitable (Servedio 2000; Sherratt 2002) and experienced bats will abort an attack in progress in response (Acharya and Fenton 1992). Some combine both acoustic and visual aposematism to enhance predator learning (Ratcliffe and Fullard 2005). For example, *Cycnia tenera* is brightly colored and produces clicks, most likely to ward off both auditory and visual predators, such as birds. However, the distinguishing characteristic between aposematic and jamming moths appears to be an increased sound production capability. One of the most distinguishing acoustic features of *Bertholdia trigona*, a known sonar jammer, is its very high duty cycle (the amount of time a signal is "on"; discussed in more detail below). In experimental setups, bats were consistently incapable of capturing *B.*

[v] Jamming is not limited to tiger moths. Some hawkmoths (Sphingidae: Choerocampina) produce ultrasound in response to bat echolocation by stridulating the genitals (Barber and Kawahara 2013).

trigona (Corcoran *et al*. 2009).

Four hypotheses[vi] were proposed to explain how moths are able to jam bat sonar and avoid capture (Fig. 2.3). The first, the distraction hypothesis, simply states that attending to two auditory streams is too tasking for the bat (Barber *et al*. 2003), reducing their effectiveness in pursuing their moth prey. The phantom echo hypothesis, states that the bat misinterprets moth clicks as its own echoes. These additional 'echoes' would create the illusion of additional objects in the acoustic field of view (Fullard *et al*. 1979; Fullard *et al*. 1994) causing bats to potentially change trajectories to avoid clutter or attempt to capture non-existent prey items. The masking hypothesis suggests that the moth clicks act as an acoustic cloaking device (Troest and Møhl 1986; Møhl and Surlykke 1989), effectively rendering the moth invisible to the bat. Finally, the ranging interference hypothesis states that moth-generated sounds degrade the bats' ability to accurately determine the distance to the target. This is most likely due to the clicks arriving before or simultaneously with the bats returning echoes and is supported by behavioral experiments (Corcoran *et al*. 2011). This does not preclude the validity of the other hypotheses in scenarios other than the prey capture sound-producing moths; it is entirely possible that another type of signal produced in a different context could be explained by these other mechanisms.

[vi] Additional hypotheses on the role of moth clicks suggest that moth sounds may startle bats (Edmunds 1974; Hoy et al. 1989). We consider this to be very unlikely after initial encounters. Bats habituate to new sounds (Bates and Fenton 1990; Miller 1991) and post-habituation effects of jamming stimuli have been confirmed (Corcoran et al. 2011). Also, the clicks may function in acoustic aposematism (Dunning and Roeder 1965; Acharya and Fenton 1992) or mimicry (Barber and Conner 2007; Barber *et al*. 2009) to advertise potential unpalatability (Weller *et al*. 1999; Hristov and Conner 2005b).

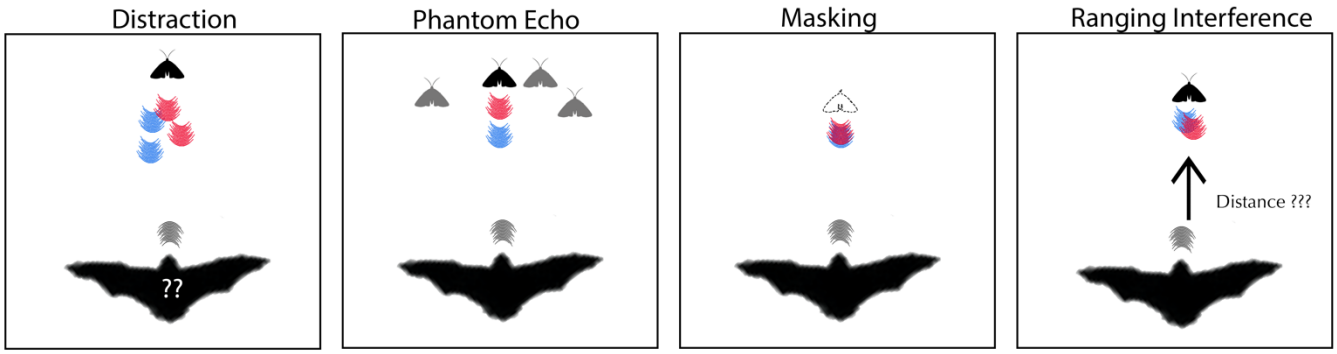

Figure 2.3. Four primary hypotheses on the mechanisms of sonar jamming in tiger moths. Tiger moths, such as *Bertholdia trigona* (black moth silhouettes), jam bat sonar (black sound waves) with rapid click trains (red sound waves) generated by the tymbal organ. The distraction hypothesis predicts that bats cannot attend to the echoes of the prey item (blue sound waves) because it cannot process two streams of auditory data. The phantom echo hypothesis predicts that moth clicks will create the illusion of clutter or additional prey items (gray moth silhouettes). The masking hypothesis predicts that moth clicks will drown out the bats' echoes and making it undetectable (dotted moth outline). The ranging interference hypothesis predicts that moth clicks make it difficult for bats to judge the distance between themselves and the prey item.

Studies have confirmed that both insects (Miller 1991; Tougaard *et al.* 1998; Corcoran *et al.* 2009; Corcoran and Conner 2012) and conspecifics (Corcoran and Conner 2014) are capable of producing high-frequency acoustic signals that interfere with a bats' ability to successfully localize and capture a prey item. In both circumstances, the signal is composed of FM components that share similarities to the FM portions of echolocation calls. Moth-generated sounds are generally stimulated in the later stages of an attack, namely, the terminal buzz phase of the bat call (Miller 1991). Jamming signals produced by *Tadarida brasiliensis* targeted at competing conspecifics are assumed to similarly disrupt processing, causing a jammed bat to miss its target and allowing the jamming individual an opportunity to capture the prey item for itself[vii]. These jamming signals differ from normal calls of conspecifics in that they are directed toward the receiving bat and are structured as a sinusoidal FM (sinFM; Fig. 2.4) call (Corcoran

[vii] Alternatively, it has been proposed that these sinFM calls function more like the food claiming calls documented in *Eptesicus fuscus* (Wright et al. 2014). When the study was replicated in captive bats, a decrease in performance was not observed, but there were significant changes in echolocation (Jones et al. 2018).

and Conner 2014). Active jamming signals are thought to be most effective when they arrive within a 2 ms window prior to the onset of a returning echo (Surlykke and Miller 1985; Miller 1991; Tougaard *et al*. 2003). Filling 10% of these critical windows is sufficient to degrade the ranging precision of bats (Miller 1991) and *B. trigona* has been shown to fill up to 85% (Corcoran *et al*. 2011). Thus, those animals that produce numerous clicks are most likely to be successful in jamming bat echolocation.

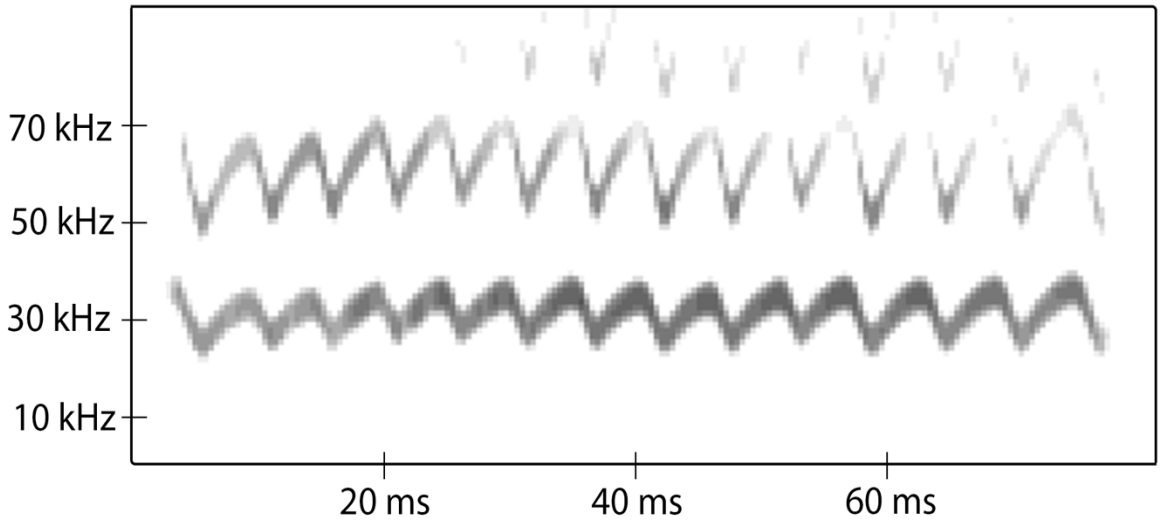

Figure 2.4. Example of a sinusoidal FM call of *Tadarida brasiliensis*.

Neurons in the auditory regions of the bat brain respond preferentially to the direction of FM sweeps (Fuzessery *et al*. 2011), suggesting that frequency modulated portions of the signals may play an important role in jamming. When presented with an interfering click before or during an FM sweep, the neuronal response of the sweep-sensitive neurons in the *inferior colliculus* (IC) and *lateral lemniscus* was partially or completely suppressed in approximately 25% of the neurons (Tougaard *et al*. 1998). Constant latency neurons in the *lateral lemniscus* are thought to play a role in coding pulse-echo delay (Covey and Casseday 1991; Covey and Casseday 1995), and thus are expected to have a role in the ranging capabilities of the bat. In

Eptesicus fuscus, a click presented before or during a tone or FM sweep interfered with the latency of these neurons (Tougaard *et al.* 1998), which could potentially interfere with bats' ability to locate prey.

III. AVOIDING ACOUSTIC INTERFERENCE: JAMMING AVOIDANCE RESPONSES

Donald Griffin was the first to describe the flexibility of bat echolocation and was the first to suggest that bats were potentially unaffected by sounds meant to interfere with echolocation (Griffin *et al.* 1963), most likely due to the ears of bats providing additional directional information that is useful in avoiding misinterpreting the calls of another bat as one's own.

III.I. Avoiding passive jamming signals

When faced with abiotic sounds, such as running water, one would predict that bats would simply avoid such an acoustically unfavorable environment. *Myotis* demonstrate behavior consistent with this hypothesis by avoiding flying near running water when foraging (Frenckell and Barclay 1987; Schaub *et al.* 2009). Bats also make use of spatial memory (Griffin 1958; Mueller and Mueller 1979; Barchi *et al.* 2013) when flying in familiar areas, reducing the number of echolocation calls needed to adequately navigate.

Interference from the calls and echoes of conspecifics, unlike background sources of noise, cannot be avoided by simply moving to a new location and is more difficult to elude due to the gregarious nature of bats. However, there are several built-in features of the echolocation system that have the potential to allow bats to readily overcome this type of passive acoustic interference.

Individual bats may already use signals that are different enough not to interfere with one another (Chiu *et al*. 2009), and individual differences have been documented (Masters *et al*. 1995; Kazial *et al*. 2001a; Siemers *et al*. 2005; Siemers and Kerth 2006).

It has been suggested that numerous echoes are required to form an accurate acoustic representation of the environment (Moss and Surlykke 2001) and, if true, the interruption of a few of these echoes should not drastically alter a bats' perception, though this has yet to be empirically demonstrated.

There also exists a high degree of directionality in both the echolocation emissions (Schnitzler and Grinnell 1977) and the sensitivity of the ears (Grinnell and Schnitzler 1977). This means a bats' own echoes (likely returning from straight ahead) should not be affected by the occasional acoustic disturbances arising from other directions around the bat. Recently, a study conducted by Adams *et al*. (2019) demonstrated that the direction of an interfering signal is more important than amplitude. Regardless of amplitude, signals which were broadcast from behind the bat induced a greater reduction in call rate than signals that were broadcast at the bat from straight ahead, even if that signal was much louder than the bats' own echolocation calls. This suggests that bats are most susceptible to interfering signals from conspecifics that may be flying along the same axis and in the same direction.

III.II. Avoiding active jamming signals

Active jamming signals, such as those produced by insects in response to bat echolocation or competing conspecifics, present a different challenge. As these signals must be designed to counteract all the aforementioned solutions to overcoming acoustic interference, we would expect, too, see some other countermeasure in the bats' repertoire. This comes in the form of a

behavioral jamming avoidance response (JAR) in which bats change the acoustic parameters of the their biosonar.

Just as in the weakly electric fishes (WEF) discussed previously, there are two categories of JAR: spectral and temporal. By definition, a spectral JAR should result in the shifting of frequencies away from those of the stimulus, whether it is another individual or a broadcast recording. Considering the JAR employed by different classes of WEF (pulse vs. wave species), we would expect this to be the JAR utilized by CF-FM bats with long, narrowband calls. A temporal JAR should strive to reduce the temporal overlap of emitted signals and this would, in theory reduce the temporal overlap of the interfering stimulus with returning echoes. Bats will also only pay attention to echoes that return within a certain time window after emitting a call. Thus, patterned calling in combination with the temporal analysis window could allow for accurate processing. Since pulse-type WEF species use this JAR, we would expect this to be the one employed by FM bats emitting many short pulses of sound.

Numerous studies have demonstrated that bats can alter almost every acoustic parameter of their calls and which changes are employed appear to be highly context-dependent (Masters *et al.* 1995; Pearl and Fenton 1996; Kazial *et al.* 2001a; Fenton *et al.* 2004; Siemers and Schnitzler 2004; Yovel *et al.* 2009). Table 2.1 outlines all the studies considered here and their results. For spectral examples, we see bats that will bidirectionally shift their frequencies away from a CF tone (Ulanovsky *et al.* 2004; Bates *et al.* 2008; Gillam and Montero 2016), shifting frequencies up or down to achieve less spectral overlap (Ulanovsky *et al.* 2004), and altering their peak frequencies, possibly to maximize individual differences (Ibáñez *et al.* 2004; Ratcliffe *et al.* 2004; Bartonička *et al.* 2007; Necknig and Zahn 2011; Corcoran and Conner 2014). In some cases, such as *Eptesicus fuscus*, one bat in a pair will cease echolocating altogether for short periods (Chiu *et al.* 2008).

Temporally, bats have been observed to alter their emission rates (Jarvis *et al*. 2010; Jarvis *et al*. 2013; Adams *et al*. 2017), duration (Obrist 1995; Gillam *et al*. 2007; Tressler and Smotherman 2009; Corcoran *et al*. 2011; Amichai *et al*. 2015; Cvikel *et al*. 2015), and intensity when other bats are nearby (Schmidt and Joermann 1986; Amichai *et al*. 2015). Takahashi *et al*. (2014) demonstrates that bats alter the timing of their pulse emissions not to coincide with jamming signals, similar to the JAR we see in pulse-type WEF. Although some of these studies claim to support bats exhibiting a JAR, others suggest the changes do not strictly fit into the definitions (Ulanovsky *et al*. 2004; Amichai *et al*. 2015; Cvikel *et al*. 2015).

This variability of responses can be attributed to several factors. First, studies may be conducted in the lab or in the field and the bats may simply be flying or foraging together, or may hear a stimulus broadcast by the researchers. These stimuli range from white noise and pure tones to altered versions of actual bat calls. Second, there are many different bat species and they differ in their echolocation types, habitats, and mode of foraging (i.e. aerial hawking vs. gleaning). Some bats also exhibit different echolocation call adaptations when they are flying with conspecifics vs. heterospecifics (Necknig and Zahn 2011). Third, the types of tasks bats perform vary and adaptations in spectrotemporal characteristics of echolocation calls appear to depend on the task at hand (Griffin *et al*. 1960; Obrist 1995). In some studies, acoustic recordings of echolocation calls are taken as bats fly together in an enclosed space or as they emerge from a roost site, compete for a prey item, avoid obstacles, or not fly at all and instead participate in forced choice tests. Finally, not all researchers measured the same set of parameters. Spectral components, such as maximum, minimum, and peak frequencies and total bandwidth are commonly reported, but some researchers report only temporal features, such as call duration or rate. Other features, like intensity, are difficult to measure without the proper calibrated

equipment and very few studies take this measurement. Some researchers report only search phase calls, while others include the approach, and only one, as far as we know, has considered changes in the terminal buzz, although changes in this phase have been linked to task complexity (Hulgard and Ratcliffe 2016).

Many of the characteristics that would allow FM bats to overcome interference, such as short duration calls across with large bandwidth, are not present in the calls of CF-FM bats. However, one of the most noteworthy observations is that the echolocation of CF-FM bats is difficult to disrupt. Rather than alter frequency as expected, these bats may alter their intensity (Schmidt and Joermann 1986). For CF-FM bats, their hearing is most sensitive at specific frequencies and emit echolocation calls whose echoes will return at those frequencies (Neuweiler 2000). The auditory neurons of CF-FM bats are also specifically tuned to the bat's own "personal" sound frequency in a spectral band referred to as the "acoustic fovea" (Suga *et al.* 1987), and it may be difficult to interfere with their echolocation using other frequencies. Naturally occurring jamming sounds are often composed of frequency modulated components, making them more distinct from a CF-FM bat's echolocation than a FM bat's calls.

IV. Alternatives to the traditional JAR

An active jamming avoidance response may not be the only explanation for why bats change their echolocation parameters in the presence of sonar interfering sounds. There is a myriad of reasons that a bat may change its echolocation patterns. There are currently two primary alternative hypotheses that could offer an explanation, depending on the type of interference being presented.

IV.I. The Lombard Effect

Étienne Lombard, a French otolaryngologist, first discovered the Lombard effect in 1911 (Lombard 1911). This phenomenon is present in many birds (Cynx *et al.* 1998; Brumm and Todt 2002; Kobayasi and Okanoya 2003), frogs (Love and Bee 2010; Shen and Xu 2016), and mammals (Sinnott 1975; Nonaka *et al.* 1997; Brumm 2004; Scheifele *et al.* 2005; Roian Egnor and Hauser 2006). In humans, this is generally a change in vocal production when conversing in noisy environments. While it can be controlled (Pick *et al.* 1989), it is generally regarded as a simple reflex (Junqua 1996). The most notable change is an increase in vocal amplitude but is also often accompanied by changes in vowel duration and intensity (Summers *et al.* 1988; Junqua 1996) and changes the first formant frequency (Garnier and Henrich 2014). These changes are collectively termed 'Lombard speech.'

The significance of this discovery is not just the production of intelligible communication, but also that these changes in speech are the result of a feedback system between vocal production and auditory perception. This feedback system is critical to the self-correction of speech required (Zollinger and Brumm 2011). While speech is an important communication tool between people, for bats auto-communication determines survival. In fact, audiovocal feedback is so important for bats that the neural circuits that control it function as early as two weeks of age and continue to mature with time (Luo, Lingner, *et al.* 2017). All the neuronal substrates responsible for the effect are not completely known, although in mammals they are believed to reside in the brainstem (Nonaka *et al.* 1997; Hage *et al.* 2006).

This particular change could be considered an extension of the traditional JAR, rather than a completely different alternative. And although it is typically referred to in the context of white noise being the source of interference, it could potentially occur in response to any given

stimulus. (Kalko and Schnitzler 1993). In bat-speak, this phenomenon typically manifests as an increase in call amplitude in the presence of acoustic interference along with increases in frequency (Hage *et al*. 2013), signal duration and number of calls (Luo *et al*. 2015). Bats are thus required to observe the ambient noise level on a continuous basis to be able to achieve these changes and their responses occur nearly instantaneously with only a 30 ms latency (Luo, Kothari, *et al*. 2017). This phenomenon also raises the question of whether the underlying neural mechanism responsible for jammed sonar has to do with interrupting the audiovocal feedback loop, making it difficult for bats to form internal templates of their calls to which they can compare returning echoes (discussed in (Corcoran and Moss 2017)). Currently, it is thought that jamming signals interfere with echo detection and that changes in echolocation are to make echo returns more distinct from the jamming stimuli. However, it should be considered a possibility that jamming signals make echo comparison difficult which would result in similar behavioral changes like decreases in performance while performing tasks such as obstacle avoidance and foraging.

IV.II. Sonar-Guided Attention

Among the myriad of reasons that a bat may change its echolocation is that objects entering the acoustic "field of view" draw the attention of the bat away from some other task. Objects may be background clutter such as buildings or trees, or the presence of other bats flying nearby. Even in laboratory studies, it can be difficult to determine to what exactly the bat is directing its attention.

In some studies, bats always increase their echolocation frequency in response to acoustic interference, even if this increase does not result in less spectral overlap with the source of the

noise (Amichai *et al*. 2015; Cvikel *et al*. 2015). If we accept the strict definition from studies on WEF that a JAR must reduce the spectral or temporal overlap of signals, then the observed changes in some echolocating bats must attributed to some other cause.

The attention hypothesis stems from research noting that bats frequently change their echolocation calls, dependent upon many factors in the environment. Bats may truncate their buzz phase when performing a landing task as opposed to a foraging task (Melcón *et al*. 2007), supported by studies that name task complexity as the cause (Hulgard and Ratcliffe 2016). In complex navigation tasks, bats may increase bandwidth to reduce errors associated with Doppler shifts occurring as a function of flight (Holderied 2006), which can impact ranging performance. The increased bandwidth helps to avoid such ranging errors, but only within the "distance of focus," a short-range, high-acuity zone in which a bat can more accurately separate a target from background clutter (Jones and Holderied 2007b). They may also decrease pulse duration to avoid pulse-echo overlap by shrinking the signal overlap zone, the area around the bat where forward masking occurs (Kalko and Schnitzler 1993).

IV.III. Signal-to-Noise Ratio

Multiple studies have converged on the idea that traditional JARs nor the above alternatives provide adequate explanation of the observed variability in bat echolocation when presented with jamming signals (Ulanovsky *et al*. 2004; Chiu *et al*. 2008; Amichai *et al*. 2015; Cvikel, Levin, *et al*. 2015; Götze *et al*. 2016; Jones *et al*. 2018). One final alternative we propose is the signal-to-noise ratio (SNR) hypothesis, which encompasses the traditional JAR along with any other echolocation changes that may be observed. These changes differ in that they are designed to facilitate optimal signal transmission, i.e. receiving an echo, by maximizing SNR. In

other words, the bats will change their echolocation as needed to increase the detection of target echoes over the various sources of acoustic interference present in the environment.

We consider this the most viable explanation, because it means bats show flexibility in their echolocation responses dependent upon the qualities of particular signals. This plasticity can also account for the vast array of audiovocal responses reported in the literature when different bat species are studied using a variety of signals in experimental setups.

It is unlikely, however, that echolocation feature adjustments alone can account for bats' ability to avoid sonar jamming. Rather, bats likely make use of several mechanisms to overcome the diversity of interfering signals they encounter. These include, but are not limited to, directional beam emissions and pinna reception, a small window in which to process returning echoes (discussed briefly in section III.II), and the sequential analysis of echoes (Greiter and Firzlaff 2017). I argue that any behavioral change can be classified as a jamming avoidance response if its product is the reduction of the effects of acoustic interference. This allows for a formal definition with specific parameters, while also allowing for the extreme flexibility of echolocation within and between bat species. This would mean that everything from increasing the distance between the source of a noise (for example: Schaub *et al*. 2009) to the various echolocation changes outlined in Table 2.1 all constitute a JAR and the JAR utilized is dependent upon the environment, task, and species-specific constraints of echolocation. In most cases, these changes can also serve to increase signal-to-noise ratio in order to optimize signal transmission; that is, bats adjust their echolocation call features as needed to enhance processing of selected auditory objects.

V. Conclusion

For nearly 80 years, active sensing has been the subject of intense study in multiple vertebrate systems. These organisms have not only provided insight into the functions, underlying physiological mechanisms, and evolution of these systems, but have also established that these systems can be disrupted and that disruption can be counteracted by changing system parameters. But there are also intrinsic parameters to individual signals that make them more recognizable to the sender/receiver, thus reducing mix-ups with those from other individuals.

Although echolocation calls are very similar among members of the same species, individuals do show distinct features that give them a unique "voice" (Masters *et al.* 1991; Masters *et al.* 1991) and are capable of distinguishing their own calls from those of other bats (Yovel *et al.* 2009). Individual variation in calls is speculated to enable individuals to tell each other apart and gather important information, such as sex (Kazial and Masters 2004), and also serve to help reduce the passive interference of nearby conspecifics during echo processing. However, what intrinsic parameters make an individual bats' signal unique continues to evade researchers.

Any system dependent upon the use of sonar must minimize interference from other acoustic sources. Thus, overcoming the negative effects of sonar interference is not only relevant to those who study echolocating animals, but also to those who design sonar, radar, and lidar technologies for military and civilian applications. With more and more of these systems being placed into everyday use, such as those that help self-driving cars to be fully autonomous, solving the problem of interpreting individual signals has

applications to understanding nature and improving insights into engineering for human application.

Table 2.1. **Comprehensive list of jamming avoidance studies.** Summaries of all studies considered in the evaluation of bat jamming avoidance response (JAR)* including the bat species and echolocation type and results. This is meant to give a broad overview of the major findings of numerous papers on the topic of jamming and jamming avoidance in bats. It is also important to note that authors may use different methods for obtaining spectrotemporal parameter values and care should be taken if making direct comparisons. Abbreviations are as follows: F_{max} = highest frequency in a call, sometimes referred to as start frequency in FM bats, denoted as F_{start}; F_{min} = lowest frequency in a call, sometimes referred to as end frequency in FM bats, denoted as F_{end}; F_{bw} = total bandwidth of a call; F_{peak} = the peak frequency of a call; F_{call} = the dominant frequency used by CF-FM bats during the CF portion of the call.
*Not all studies were originally conducted in an effort to quantify bat JARs.

							JAR			
Species	**Dominant Echolocation Type**	**Study Site**	**Number of bats**	**Source of interference**	**Type of interference**	**Task**	**Spectral**	**Temporal**	**Intensity**	**Author/Year**
Lasiurus borealis	FM	Field	Pairs	Bat (Conspecific)	Passive	Free Flight	Increase: F_{start}, F_{peak}	Increase: IPI Decrease: call duration		Obrist 1995
Tadarida teniotis	FM	Field	Pairs	Bat (Conspecific)	Passive	Free Flight	Bidirectional frequency shifts	None		Ulanovsky *et al.* 2004
Taphozous perforatus	FM	Field	Pairs	Bat (Conspecific)	Passive	Free Flight	None	None		Ulanovsky *et al.* 2004
Tadarida brasiliensis	FM	Field	Group	Recording (Conspecific)	Passive	Free Flight	Increase: F_{call}, F_{bw}	Decrease: IPI, call duration		Gillam *et al.* 2007
		Lab	Single	Recording (Conspecific)	Passive	Free Flight	Not reported	Reduce emission rate		Jarvis *et al.* 2013
		Lab	Single	Recording (Broadband noise)	Passive	Free Flight	Not reported	Reduce emission rate		Jarvis *et al.* 2010

		Lab	Single	Recording (Broadband noise)	Passive	Stationary	Increase: F_{start}, F_{bw}, F_{peak} Decrease: F_{end}	Increase: call duration	Increase: amplitude	Tressler and Smotherman 2009
		Field	Pairs	Bat (Conspecific)	Passive	Free Flight	Greater difference F_{peak}	None		Ratcliffe *et al.* 2004
		Lab	Pairs	Bat (Conspecific) Recording (Conspecific)	Passive	Obstacle avoidance	Not reported	Reduce emission rate		Adams *et al.* 2017
		Field	Group	Recording (Conspecific)	Active	Tethered prey capture	Increase: F_{peak}, F_{min}, F_{max}	None		Corcoran and Conner 2014
		Field	Group	Recording (CF and FM sounds)	Passive	Free Flight	Increase: F_{start}, F_{end}, F_{max}	Not reported		Gillam and Montero 2016
Rhinopoma microphyllum	CF-FM	Field	Group	Bat (Conspecific)	Passive	Free Flight	Increase: F_{call}, F_{bw}	Decrease: call duration		Cvikel, Levin, *et al.* 2015
		Lab	Single	Noise	Passive	Free Flight	None	Not reported	Increase: intensity	Schmidt and Joermann 1986
Eptesicus fuscus	FM	Lab	Pairs	Bat (Conspecific)	Passive	Tethered prey capture	Increase: F_{start}, F_{end}, F_{bw}	None		Chiu *et al.* 2009
		Lab	Single	Moth Prey	Active	Tethered prey capture	None	Decrease: buzz phase duration Increase: call duration		Corcoran *et al.* 2011

		Lab	Pairs	Bat (Conspecific)	Passive	Tethered prey capture	Silence	Silence		Chiu *et al.* 2008
		Lab	Single	Recording (CF tone)	Passive	Non-flying/target discrimination	Bidirectional shift of QCF component	None		Bates *et al.* 2008
		Lab	Single	Recording (Heterospecific)	Active	Tethered prey capture	Increase: F_{peak}, F_{bw}	Decrease: sweep rate Increase: call duration		Jones *et al.* 2018
Pipistrellus abramus	FM	Lab	Single	Bat (Conspecific) Recording (Conspecific)	Passive	Free Flight	Increase: F_{end}	Alter emission timing		Takahashi *et al.* 2014
Corynorhinus (Plecotus) townsendii	FM	Lab	Single	Recording (Broadband noise)	Passive	Obstacle avoidance	Not reported	Not reported		Griffin *et al.* 1963
Pipistresllus nathusii	FM	Field	Group	Bat (Conspecific) Bat (Heterospecific)	Passive	Free Flight	Increase: F_{peak}	Not reported		Necknig and Zahn 2011
Pipstrellus pipistrellus	FM	Field	Group	Bat (Conspecific) Bat (Heterospecific)	Passive	Free Flight	Increase: F_{peak}	Not reported		

		Lab	Group	Bat (Conspecific) Bat (Heterospecific)	Passive	Free Flight	Increase: differences in F_{peak}	None		Bartonička *et al.* 2007
		Field	Group	Bat (Conspecific)	Passive	Free Flight	Increased: differences in CF-portion of call	Not reported		Miller and Degn 1981
Balantiopteryx plicata	CF-FM	Field	Group	Bat (Conspecific)	Passive	Free Flight	Increase: difference F_{peak}, F_{max}	Not reported		Ibáñez *et al.* 2004
Rhinopoma hardwickei	CF-FM	Field	Group	Bat (Conspecific)	Passive	Free Flight	Utilized 3 different CF bands	Not reported		Habersetzer 1981
Pipistrellus kuhlii	FM	Lab	Single	Recording (Conspecific) Recording (Self)	Passive	Landing on platform; Obstacle avoidance	Increased: F_{bw}	Increase: call duration (QCF portion)	Increase: intensity	Amichai *et al.* 2015
Craseonycteris thonglongyai	CF-FM	Field	Group	Bat (Conspecific)	Passive	Free Flight	None	None		Surlykke *et al.* 1993
Hipposideros speoris	CF-FM	Lab	Group	Bat (Conspecific)	Passive	Free Flight	Bidirectional shift	Not reported		Jones *et al.* 1994
	CF-FM	Lab	Single	Recording (Conspecific) Recording (Self)	Passive	Obstacle avoidance	Small scale frequency changes	Not reported		Jones *et al.* 1994

Hipposideros fulvis	CF-FM	Lab	Single	Recording (Conspecific) Recording (Self)	Passive	Obstacle avoidance	Small scale frequency changes	Not reported		
Asella tridens	CF-FM	Lab	Group	Bat (Conspecific)	Passive	Free Flight	None	Not reported		Jones *et al.* 1993
Miniopterus fuliginosus	FM	Lab	Single	Recording (FM sounds)	Passive	Free Flight	Increased: F_{end}	Not reported		Hase *et al.* 2016
Rhinolophus capensis	CF-FM	Lab	Group	Bat (Conspecific)	Passive	Free Flight	Decrease: F_{min} in terminal FM component	Increase: duration of terminal FM component	Decrease: intensity	Fawcett *et al.* 2015
		Lab	Group	Bat (Heterospecific)	Passive	Free Flight	Decrease: F_{min} in terminal FM component	Decrease: call duration Increase: duration of terminal FM component		

Chapter III

Active acoustic interference elicits echolocation changes in heterospecific bats

CHAPTER NOTES

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Associated supplemental materials can be reviewed by accessing the JEB website.

The jamming of active sensory systems has garnered a great deal of attention since the early experiments quantifying jamming and its effects in the weakly electric fishes. These works served as inspiration for similar experiments conducted on echolocating bats which has led to an expanse of literature documenting performance degradation in a variety of tasks under many types of noisy conditions. One particularly interesting discovery was the apparent sonar jamming of the aerial-hawking *Tadarida brasiliensis* by conspecifics competing for the same prey item(Corcoran and Conner 2014). In these interactions, one bat would emit a sinusoidal frequency-modulated call (sinFM) toward a bat pursuing a prey item, resulting in a significant number of failed capture attempts from the targeted bat, provided the sinFM call overlapped the terminal buzz. This is one of the few studies to focus on active jamming, referring to signals whose purpose is to interfere with echolocation, and the only study thus far that has done so with bats as the source of jamming.

Because jamming has the capacity to degrade performance during prey capture and other critical routine tasks, and because bats are frequently observed foraging in group containing both conspecifics and heterospecifics, they must be able to employ some defense against it. A relatively simple solution would be to fly elsewhere for food, but since larger groups tend to indicate a greater abundance of prey, bats would risk missing out on fruitful foraging. Instead, it has been proposed that bats exhibit a jamming avoidance response (JAR), a phenomenon first described in the weakly electric fishes. To date, there is no general agreement as to what defines a JAR in bats, whether they possess this ability at all, or, if able, it is effective in jamming scenarios. The goals of this chapter are to 1) determine if heterospecific active jamming signals are effective in degrading prey capture performance and 2) determine if heterospecific active jamming signal elicit echolocation changes that could be consistent with a JAR and if these changes effectively counteract the jamming signals.

ABSTRACT

Echolocating bats often forage in the presence of both conspecific and heterospecific individuals, which have the potential to produce acoustic interference. Recent studies have shown that at least one bat species, the Brazilian free-tailed bat (*Tadarida brasiliensis*), produces specialized social signals that disrupt the sonar of conspecific competitors. We herein discuss the differences between passive and active jamming signals and test whether heterospecific jamming occurs in species overlapping spatiotemporally, as well as whether such interference elicits a jamming avoidance response. We compare the capture rates of tethered moths and the echolocation parameters of big brown bats (*Eptesicus fuscus*) challenged with the playback of the jamming signal normally produced by Brazilian free-tailed bats and playback of deconstructed versions of this signal. There were no differences in the capture rates of targets with and without the jamming signal, although significant changes in both spectral and temporal features of the bats' echolocation were observed. These changes are consistent with improvements of the signal-to-noise ratio in the presence of acoustic interference. Accordingly, we propose to expand the traditional definition of the jamming avoidance response, stating that echolocation changes in response to interference should decrease similarity between the two signals, to include any change that increases the ability to separate returning echoes from active jamming stimuli originating from conspecific and heterospecific organisms. Flexibility in echolocation is an important characteristic for overcoming various forms of acoustic interference and may serve a purpose in interspecific interactions as well as intraspecific ones.

INTRODUCTION

In many species of echolocating bats, sonar signals are composed of frequency modulated (FM) sound pulses separated by varying lengths of silence during which the bat listens to the returning echoes. This method of active sensing using self-generated acoustic signals yields information crucial in navigating complex environments as well as detecting and pursuing prey in conditions with little or no light (Griffin 1958; Fenton 2003). However, echolocation is susceptible to acoustic interference, including sonar jamming, which can potentially disrupt foraging. Interference can come from ambient sources of noise in the environment such as running water, rustling leaves, anthropogenic noise and the high-frequency calls of insects. The solution of avoiding jamming under these circumstances is relatively effortless as the bats can choose to forage elsewhere. Yet, interference can also originate from the calls of other bats (Dusenbery 1992). In these scenarios, the solution for avoiding interference is less straightforward.

Foraging bats often 'eavesdrop' on others to find food (Cvikel, Egert Berg, *et al.* 2015) by listening for the terminal (feeding) buzzes of conspecifics, meaning that the sounds of feeding will always have the potential to attract additional individuals, even should that bat change foraging sites. When bats feed in mixed-species groups, heterospecific individuals may also cue in on feeding calls (Barclay 1982). Although bats are capable of distinguishing heterospecific calls from the calls of conspecifics (Dorado-Correa *et al.* 2013), species that share certain ecological dimensions, such as diet, may converge upon the same resources (Li *et al.* 2014). With multiple bats foraging in the same area, the acoustic background unavoidably becomes more problematic as the

potential for passive jamming rises. Adding to this complexity, Brazilian free-tailed bats (*Tadarida brasiliensis*) produce distinct social signals that actively jam the sonar of conspecific competitors, causing them to miss their targets (Corcoran and Conner 2014). Bats have been observed employing a number of tactics to avoid sonar jamming, including remaining silent while using passive sensing (Chiu *et al*. 2008) and altering various parameters of echolocation calls in real time (Gillam and McCracken 2007). Collectively, these dynamic vocal adjustments can be utilized to avoid both spectral overlap (Surlykke and Moss 2000) and temporal coincidences (Obrist 1995) in the form of a jamming avoidance response (JAR), similar to the behavior found in weakly electric fishes (Bullock *et al*. 1972). But investigators study numerous species and there is little consistency in the types of signals presented and the behavioral tasks being performed. This makes it difficult to interpret results. Measurement of potential JARs is further complicated by the fact that bats adjust their vocalizations in response to the presence of other individuals, others' vocalizations or both (Amichai *et al*. 2015). In addition to controlling for the complication of multiple bats, we herein differentiate the difference between passive and active jamming. Passive jamming may be elicited by any interfering sound in the environment, including the echolocation of other bats foraging nearby, whereas active jamming refers to those signals either generated by heterospecific and conspecific bats as an adaptation to decrease food competition or produced by sonar jamming moths (Corcoran *et al*. 2009).

We tested whether active jamming signals of bats can elicit similar behavioral changes in heterospecific individuals. Only a few studies have documented effects in heterospecifics (Bartonička *et al*. 2007; Necknig and Zahn 2011; Fawcett *et al*. 2015), all of

which document changes in free-flying bats performing no specific task, and to date, only two other studies have presented active jamming signals to bats and documented the resulting changes in echolocation (Corcoran *et al*. 2011; Corcoran and Conner 2014). This experiment will be the first to present active interference stimuli of heterospecifics to bats performing a behavioral task. We chose to use sinusoidal FM (sinFM) signals of *T. brasiliensis* as our potential jamming signal, as its effectiveness on conspecifics has been documented (Corcoran and Conner 2014). The big brown bat [*Eptesicus fuscus* (Palisot de Beauvois 1796)] was chosen as the target species because of its ecological overlap with *T. brasiliensis*. Both species share similar geographic and dietary components and have been observed foraging in the same areas (A. J. Corcoran, personal communication) and both have well documented JAR behaviors (Gillam *et al*. 2007; Bates *et al*. 2008).

We predicted that sinFM calls of *T. brasiliensis* would jam a foraging *E. fuscus*, causing failed capture attempts, eliciting a JAR, or both. To test this hypothesis, we used playback experiments presenting sinFM signals to free-flying *E. fuscus* as they attempted to capture tethered prey items. Our results illustrate that active sonar jamming signals can affect the echolocation calls emitted by heterospecific bats.

MATERIALS AND METHODS

Animals

The Wake Forest University Animal Care and Use Committee approved all procedures described herein (A16-127). We used three wild-caught, adult male big brown bats (*E. fuscus*) captured near Wake Forest University (Forsyth County, NC, USA) under

NC state collecting permit 16-SC01070. The bats were housed together in cages in a temperature-controlled room (~25°C) on a 12 h:12 h light:dark cycle. Bats had continuous access to water and were fed mealworms (*Tenebrio molitor* larvae) and adult female greater wax moths (*Galleria mellonella*) nightly. Moths were acquired as larvae from King's Wholesale Bait (Liberty, IN, USA) and reared to adulthood. Individual bats were trained to remove food from a tether (described below) prior to starting playback experiments.

Experimental setup

Trials took place in an outdoor flight cage (18 m long, 5.5 m wide, 3 m tall) adjacent to Winston Hall on the Wake Forest University campus (Fig. 3.1). *Galleria mellonella* were deafened by ablating their tympanic membranes. They were tethered by the abdomen to the ceiling of the flight cage with a single monofilament line (1 m long, 0.38 mm diameter) that allowed them a limited flight radius. The flight cage was illuminated with three Raytec Raymax 200 platinum infrared illuminators (Ashington, UK).

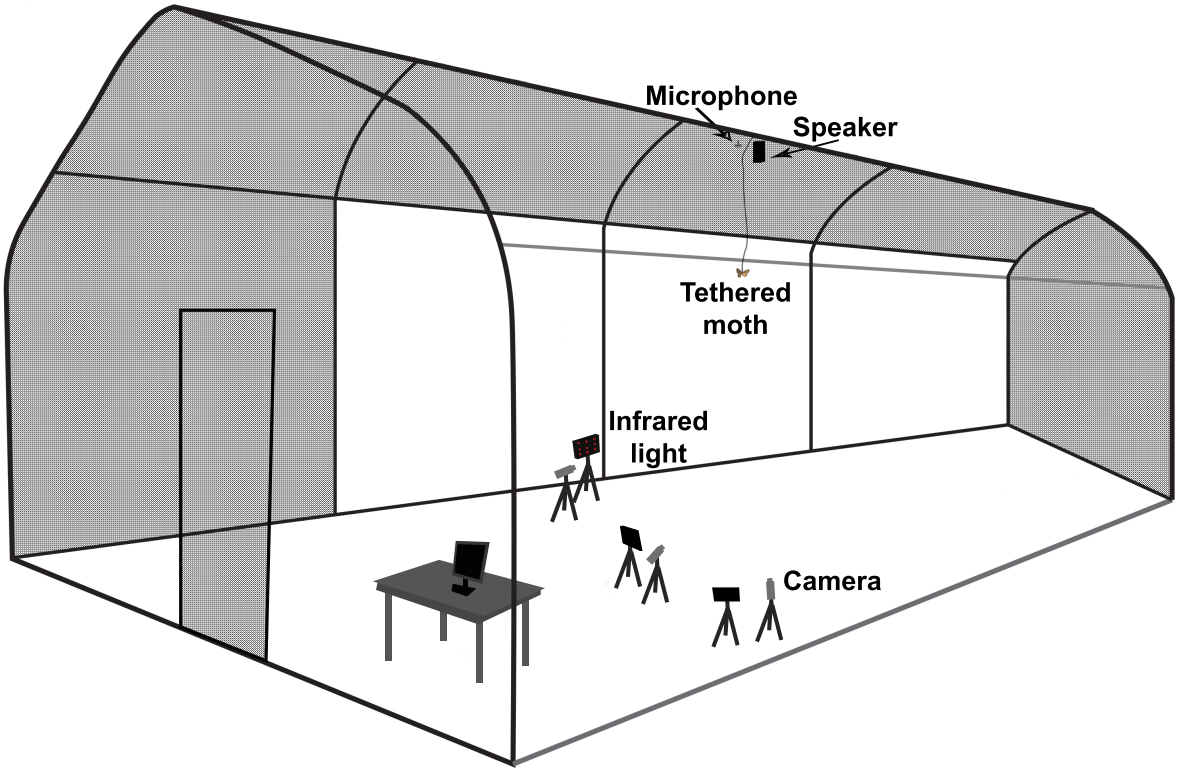

Figure 3.1. Diagram of recording setup in the mesh-covered flight cage.

Bat stimuli and playback

SinFM calls previously recorded from *T. brasiliensis* (sample rate=300 kHz) were deconstructed to include only their downsweep (n=27) or upsweep (n=30) components (Fig. 3.2). We included these deconstructed signals as a way of determining whether directionality of a FM signal influences its effectiveness, as measured by successful captures and/or alterations in echolocation parameters. Given that bats possess neurons that respond selectively to sweep direction (Suga 1968; Razak and Fuzessery 2006; Andoni *et al*. 2007; Voytenko and Galazyuk 2007), we were interested whether the presentation of these different stimuli would result in observable behavioral changes. These signals, along with the full sinFM signal (*n*=57) were played back to three bats individually during

capture attempts via an AT100 ultrasonic transmitter (Binary Acoustics Technology, Tucson, AZ, USA). The transmitter was placed 1 m above the moth and sinFM signals were produced at 95 dB SPL, measured at the position of the moth (RMS) with a Brüel and Kjær (B&K, Nærum, Denmark) ¼ inch microphone connected to a B&K 2610 amplifier. Playback was triggered manually as the bat approached the target and playback of the signal occurred continuously until the bat made contact with the target. The timing (during the search or approach phase of the echolocation sequence) was determined post-recording. The control condition was silence (n=129).

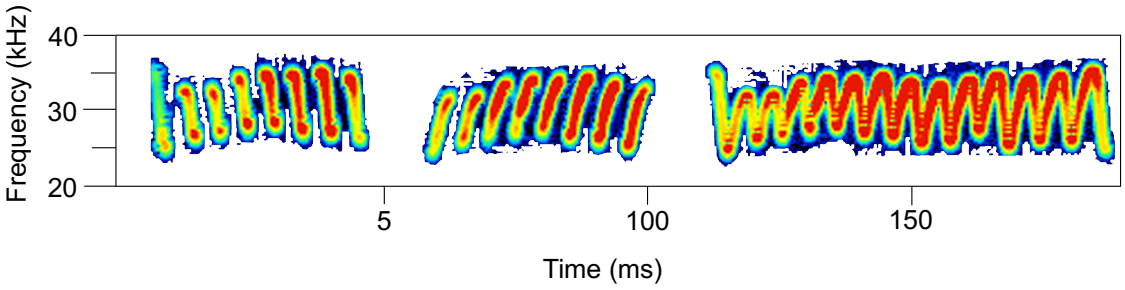

Figure 3.2. Sinusoidal frequency-modulated signal deconstructed to only the downsweep or upsweep components compared with the full sinFM. The relative amplitude of frequency content is indicated by color variations; warmer colors indicate higher amplitude.

Video and audio recording

Each trial was recorded with three calibrated high-speed, infrared-sensitive cameras (Basler Ace acA-2000-50gmNIR; Ahrensburg, Germany). Video recordings were acquired with StreamPix6 software (Norpix, Inc., Montreal, Canada) at 80 frames s^{-1} with 1280×720 pixel resolution. The echolocation calls were recorded for each trial with a small (3 mm diameter) ultrasonic microphone placed 1 m above the moth and connected to an Avisoft USGH recording unit (Avisoft Bioacoustics, Glienicke, Germany), sampling

at 250 kHz. Cameras and microphones recorded in synchrony, triggered via a TTL pulse generated with custom hardware (Innovation Systems, Columbiaville, MI, USA).

Video recordings were reviewed and categorized as (1) successful capture (n=184; Movie 1); (2) attempted capture, unsuccessful (*n*=59; Movie 2); or (3) no attempted capture or aborted attack (n=42; Movie 3). Aborted attacks were differentiated from attempted captures by the physical behavior of the bat. In an attempted capture, bats would curl the tail or wing membranes to capture the moth, regardless of whether they made contact with the moth. Those that made no attempted capture would fly by the moth without appearing to slow or displaying any changes in body conformation that would indicate capture. In aborted attacks, bats would reduce their speed and/or change directions completely, avoiding all contact with moths. Trials in which bats simply flew past the tether were not scored. Often, bats would make multiple attempts following a failed initial attempt and be successful; however, only the first attempt was evaluated in every trial. Only trials that were scored as a 1 (*n*=184) or 2 (*n*=59) were included in the analyses.

Audio recordings were reviewed, and only those in which the sinFM signal was triggered prior to, and played throughout the duration of, the terminal buzz were included. These audio files were then edited in Adobe Audition v. 5.0.2 (Adobe Systems, Inc., San Jose, CA, USA) to isolate the echolocation calls of the first capture attempt, verified with the timing of the synchronized video recordings, by manually removing the echoes of individual calls, the second and third harmonics, and the stimuli. Post-processing, parameter values were taken from the automatic parameter measurement tool in Avisoft. Files were high- and low-pass filtered at 15 and 200 kHz, respectively. The

duration threshold was set to be approximately 1 ms and frequency thresholds were set to be −38 dB. We also chose to only analyze the primary, or first, harmonic. For the purpose of this study, bat attack phases were determined based on the inter-pulse interval (IPI) of the echolocation calls and are defined as: <5–12 ms (buzz), 12–49 ms (approach) and ≥50 ms (search). A list of all measured parameters along with their abbreviations and definitions can be found in Table 3.1.

Table 3.1. Acoustic parameters. Parameters measured from each audio recording along with the abbreviations used throughout this paper and its definition.

Acoustic Parameter	Abbreviation	Definition
Pulse duration	D_{call}	Duration of individual sonar emissions
Maximum frequency	F_{max}	Highest frequency (kHz) of a sonar emission
Minimum frequency	F_{min}	Lowest frequency (kHz) of a sonar emission
Peak frequency	F_{peak}	Frequency (kHz) with the most energy in a sonar beam
Bandwidth	F_{bw}	Range of frequencies covered in a sonar emission
Inter-pulse interval	*IPI*	Time (ms) between successive sonar emissions
Sweep rate	*SR*	Quotient of bandwidth+pulse duration; describes the slope of a frequency modulated call

Statistical analysis

We first analyzed the video trials to determine whether the playback of sinFM signals resulted in more failed capture attempts than in silent conditions using a generalized linear mixed model (GLMM) with a binomial error distribution. We next examined the echolocation calls emitted by each bat during their capture attempts in order to determine whether the signal playback would result in the spectral or temporal changes associated with JAR behaviors. From each audio recording, we extracted the following parameters for bat calls in the approach and buzz phases: maximum frequency (F_{max}), minimum frequency (F_{min}), bandwidth (F_{bw}), peak frequency (F_{peak}), IPI, pulse

duration (D_{call}) and sweep rate (SR) (Table 3.1). Measurements for F_{max}, F_{min}, F_{bw}, F_{peak} and SR were taken from the spectrogram (FFT length=512, Hamming window) using SASLab Pro (Avisoft Bioacoustics). Measurements for IPI and D_{call} were taken from the oscillogram using MATLAB (The MathWorks, Natick, MA, USA). Data for each parameter were placed into a different linear mixed model (LMM). To compensate for multiple comparisons, the resulting *p*-values were adjusted using the Benjamini–Hochberg method.

All data analysis was conducted in R v. 3.3.2 (https://www. r-project.org/) using the lme4 package (Bates *et al.*, 2015). For all statistical models, individual bat identification was used as a random effect to account for the lack of independence in using individuals for multiple trials over the course of several nights. For the LMMs, the individual file containing the series of bat calls for one trial and the day on which trials were conducted were also used as separate random effects. Post hoc analyses were conducted with the lsmeans package (Lenth, 2016) and multiple comparisons were corrected with the Bonferroni-based false discovery rate method (α=0.05).

RESULTS

We found no significant difference in the proportion of successful captures between each stimulus condition ($F_{(3,236.08)}$=0.73, p=0.53). Regardless of stimuli, the bats had a high rate of successful captures, always surpassing 60% (Table 3.2). For the echolocation parameters analyzed, we present only those that were found to be statistically significant; all other data can be found in Fig. S1.

Table 3.2. The percentage of successful captures under each playback condition across all nights.

Stimulus	Successful captures (%)
Silent	78
Downsweep only	67
Upsweep only	70
SinFM	77

There was a significant increase between D_{call} in silent conditions and the playback stimuli in both the approach ($F_{(3,229.15)}$=6.94, p<0.001) and the buzz phase ($F_{(3,234.94)}$=3.78, p=0.026). There was a significant decrease in F_{bw} during the buzz phase ($F_{(3,238.18)}$=5.42, p=0.0035), and the buzz phase also showed a significant increase in F_{peak} ($F_{(3,229.71)}$=5.68, p=0.0031).

We also determined that SR of both phases was significantly decreased in playback conditions (approach: $F_{(3,226.89)}$=10.10, p<0.001; buzz: $F_{(3,230.06)}$=8.24, p=0.0022). Post hoc analyses reveal that the full sinFM signals consistently elicited longer D_{call} (Fig. 3.3A), lower F_{bw} (Fig. 3.3B) and lower SR (Fig. 3.3C) than other conditions. F_{peak} was significantly increased compared with the silent condition for all jamming stimuli (Fig. 3.3D).

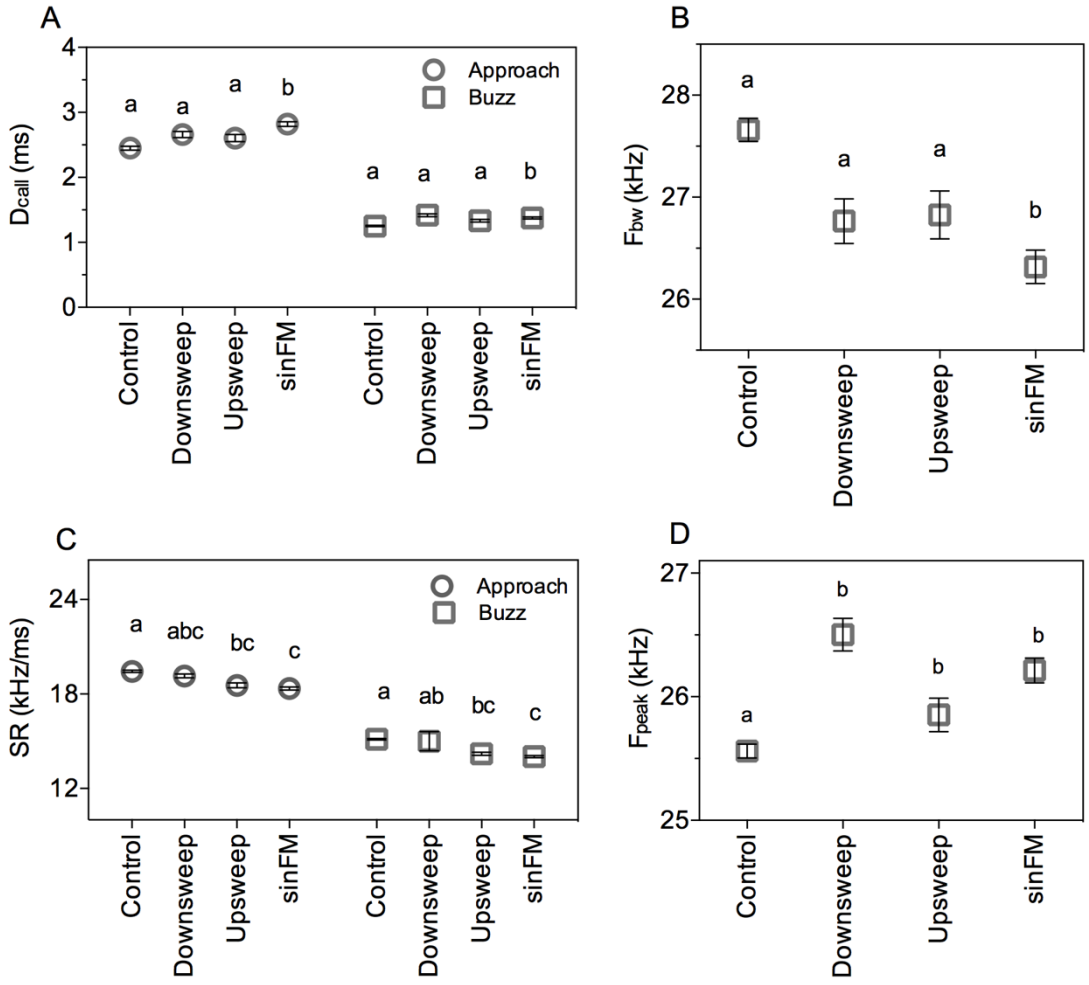

Figure 3.3. Echolocation parameters that were significantly altered in response to playback. (A) Differences in pulse duration (D_{call}) among stimuli for both the approach and buzz phases. (B) Effects of stimulus type on bandwidth (F_{bw}) of the buzz phase. (C) Differences in sweep rate (SR) by stimulus type for both the approach and buzz phases. (D) Effects of stimulus type on peak frequency (F_{peak}) of the buzz phase. Groups sharing a letter are not significantly different (false discovery rate *post hoc* test, $p<0.05$). All data are means±s.e.m.

Discussion

We predicted that *E. fuscus* would fail to capture prey items and/or exhibit echolocation changes to counteract the effects of our jamming stimuli. The bats were able to catch the prey item under all of our test conditions; thus, we did not find evidence to support heterospecific jamming capabilities. We would like to acknowledge that this could potentially be attributed to the setup of our experiment, in which the prey items were tethered and restricted in their ability to fly away, possibly minimizing the overall effectiveness of the stimuli. We did, however, show that four parameters – D_{call} (approach and buzz), F_{bw} (buzz), F_{peak} (buzz) and SR (approach and buzz) – were significantly altered between silent conditions and the different playback stimuli.

Under the strictest definition of JARs, spectrotemporal changes should increase the differences between the bats' emitted signal and the jamming stimulus (Ulanovsky 2004). We suggest expanding the definition of a JAR to include all changes that may increase the signal-to-noise ratio, as well as those that maximize spectral or temporal differences between the bats' own calls and active jamming stimuli originating from conspecific and heterospecific organisms. The term was first applied to the behaviors of weakly electric fishes and it included only the reflexive shifting of frequency, or change in timing, of electric pulses to increase disparity between two individuals (Bullock *et al.* 1972). This definition most likely encompassed such a limited range of changes because the only natural scenario in which these animals face interference is in the presence of other electrogenic fish. Because bats can encounter interference in their dominant sensing modality from numerous sources and can alter many more parameters of their signal, the

traditional usage is far too constrained to account for the all of the possible responses to these sources. Additionally, it is unclear whether the myriad of changes observed in bat echolocation can be attributed solely to reflexive shifts in response to stimuli, as we do not see evidence of stereotyped responses to even the same type of stimulus across studies. The traditional definition would also require the presence of another bat, but we already acknowledge that jamming signals can have alternate origins, such as sonar-jamming moths (Miller 1991; Corcoran *et al*. 2011). Ultimately, this would mean that all changes in signal design in response to the presentation of active jamming stimuli generated from conspecifics and heterospecifics that have the potential to improve echo reception would be considered a JAR in bat species.

The nature of echolocation is to derive meaningful information from subtle shifts in spectrotemporal characteristics, and it is likely that these fine-scale adjustments in call design can significantly impact the perception of echoes and influence task performance. The absolute changes we observed in the bats' pulse duration and sweep rates were modest, varying by approximately 1 ms or less. Other studies utilizing *E. fuscus* have shown that they are capable of changing many other echolocation parameters depending on the task being performed. These include altering start and end frequencies and bandwidth (Chiu *et al*., 2009), as well as increasing call duration and shortening the duration of the buzz phase (Corcoran *et al*., 2011) and ceasing echolocating altogether (Chiu *et al*., 2008). Many of these changes were also small scale, on the order of a few kilohertz and or milliseconds, just like our observations.

Bats responded differently to each of the stimuli presented. Playback of the full sinFM resulted in the most changes in echolocation parameters and we consider it to be

the most effective at eliciting changes. Upsweep-only and downsweep-only signals elicited similar responses. These results are somewhat counterintuitive, as it was expected that signals most similar to the bats' own calls, the downsweep-only signals, would be most effective. Evidence to the contrary could possibly be due to bats utilizing templates of their own calls for echo recognition. It has been proposed that bats compare the time–frequency structure of their call emissions with that of the returning echoes (reviewed in Corcoran and Moss, 2017). *Eptesicus fuscus* presented with upward-sweeping FM calls (Masters and Jacobs, 1989) or stimuli otherwise altered in time or frequency (Masters and Raver 1996; Masters and Raver 2000) displayed reduced abilities in range discrimination tasks. These studies suggest that bats are most capable of extracting information from calls that are most similar to their own templates.

Although this provides a feasible argument as to why downward-sweeping FM calls require the least compensation, it is still difficult to determine how all of these stimuli provoked changes in echolocation. However, we can evaluate the potential advantages these changes provide. FM calls are best for determining target range and structure as the increased bandwidth increases the resolving power of the call (Holderied 2006). Though we observe an approximately 1 kHz downshift in bandwidth in the buzz phase, we argue that bats could afford this potential sacrifice in resolution because of the high information redundancy of an increased pulse rate typical of shifting from approach to buzz (Ratcliffe *et al*. 2013). Bats may also decrease bandwidth to concentrate more energy into fewer frequencies in order to increase the signal-to-noise ratio. The changes in peak frequency are most often attributed to increasing the differences between an individual bat and surrounding bats (Ibáñez *et al*. 2004; Ratcliffe *et al*. 2004; Bates *et al*.

2008; Necknig and Zahn 2011), or in this case, the simulated bat emitting the sinFM. However, we observed an increase in peak frequency towards that of the stimulus and believe this to be another compromise to increase signal-to-noise ratio (Tressler and Smotherman 2009). Increasing pulse durations increases signal energy, and this is thought to be a way for bats to increase the signal-to-noise ratio by increasing echo detectability (Amichai *et al*. 2015). The resulting combination of bandwidth and pulse duration, i.e. sweep rate, can thus be optimized for the echolocation task being performed.

Boonman and Ostwald (2007) used a computer model to simulate the bat cochlea responding to returning echoes and found that an optimal sweep rate is critical to the temporal resolution of multiple echoes. Sweep rate generally becomes faster as the bat closes on its target (Inoue *et al*. 2002), and this is likely to allow the bat to receive the clearest acoustic image of the target as the acuity of each echo is improved and bats are able to better determine the timing of each echo. However, faster sweep rates decrease the chances of channel activation in computer simulations, which correspond to the inner hair cells of the bat cochlea (Boonman and Ostwald 2007). Thus, decreasing sweep rates (our observation) are more likely to increase the number of individually detected echoes. This further emphasizes the sensory trade-offs bats face (Schnitzler *et al*. 2003; Ulanovsky and Moss 2008) and that these trade-offs are necessary to optimize target detection and localization.

It is important to note that not all of these parameters in which we observe changes may necessarily provide perceptible changes in returning echoes, especially given their small scale. Some changes may simply be by-products of others, but all of these

changes in various combinations may contribute something to increasing the ability to resolve returning echoes. Additionally, there are some changes we fail to see that have been documented in other studies, such as significant changes to minimum or maximum frequency. The approach calls of *E. fuscus* overlap fewer frequencies with the sinFM signal than do those of *T. brasiliensis* (Fig. 3.4), and the increased bandwidth possibly eliminates the need for any changes to further maximize differences. This could also explain why the sinFM signal did not have a negative effect on the capture success rates of *E. fuscus*. In *T. brasiliensis*, Corcoran and Conner (2014) demonstrated that playback of the sinFM resulted in a decreased number of successful captures. In response, the jammed bats only significantly increased their maximum and minimum frequencies and this did not significantly alter the overall bandwidth of the calls. Other parameters, such as call duration and IPI, were unaffected as well. This upward shift in call frequency is consistent with other studies on *T. brasiliensis* in the presence of acoustic interference. The normal echolocation calls of *T. brasiliensis* have more spectral overlap with the sinFM signal, and this shift may be an example of a traditional spectral JAR.

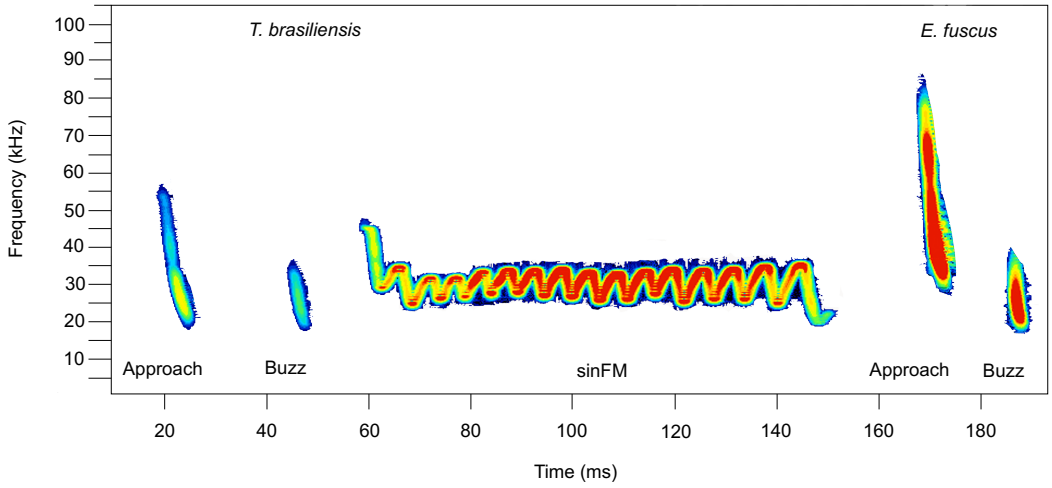

Figure 3.4. Examples of first harmonic approach and buzz calls for *Tadarida brasiliensis* and *Eptesicus fuscus* compared with the sinFM signal used in playback. There is considerably more overlap in the *E. fuscus* buzz call, and this may be why we see greater flexibility demonstrated in this portion of the signal.

However, there are two alternatives that could explain the changes observed. The first is that bats were exhibiting the Lombard effect – a physiological change in the larynx resulting in changes in intensity, often accompanied by frequency and call duration changes. This response has been documented in *E. fuscus* and results in louder calls (Luo, Lingner, *et al.* 2017). We were unable to record intensity values for the bats during their capture attempts, owing to equipment limitations, and thus, we cannot confirm or deny that they were perhaps exhibiting the Lombard effect. Because this phenomenon is generally accompanied by calls of increased duration (Takahashi *et al.* 2014; Luo *et al.* 2016) and frequency (Hage *et al.* 2013) and because our bats lengthened the duration of their calls and increased the minimum and maximum frequencies in the approach phase (though not significantly so), this is a possibility. The second explanation is the attention hypothesis that states that bats will differentially alter their echolocation based on objects in their acoustic environment drawing their attention. These objects may be various prey

items, other bats, or obstacles such as buildings or trees. Bats may shorten their pulse duration to avoid pulse–echo overlap, just as they would if they were approaching clutter (Kalko and Schnitzler 1993; Schnitzler *et al*. 2003; Melcón *et al*. 2007). This would suggest our bats were responding to the jamming stimulus as if it were an object entering their acoustic field of view, despite its purpose to reduce capture success, and this possibility has been implicated in other studies (Cvikel, Levin, *et al*. 2015; Götze *et al*. 2016). For our experiment, this is the less likely scenario, as it might be assumed that a bat shifting its attention from its current task, in this case, prey capture, would result in more failures.

Much work has been done on the subject of echolocating bats and whether they exhibit some type of response to jamming signals, though there is currently no consensus on the matter. To date, evidence for spectral or temporal JAR is somewhat conflicting as variation in echolocation is highly context dependent and experimental designs are structured with major differences. We found that in the presence of the active jamming sinFM signal of *T. brasiliensis*, *E. fuscus* significantly alters its echolocation. This is the first documentation of bat active jamming signals affecting heterospecifics and is also one of the first presentations of evidence that bats alter the structure of the terminal buzz in response to acoustic interference. Bats increased their pulse duration to increase echo detectability and decreased sweep rates to generate more accurate echo timing in both the approach and buzz phases. In the buzz phase, bats decrease their bandwidth to concentrate energy over a reduced range of frequencies and increase the peak frequency to help differentiate their calls from the stimuli. Many of these changes reflect similarities found in other JAR studies while others are novel findings. All of our observations support the hypothesis that bats are altering their echolocation to increase the signal-to-

noise ratio of perceived echoes, a JAR under our expanded definition. Though our understanding of how particular features of jamming stimuli elicit changes is still developing, it is clear that the echolocation flexibility of bats allows them to remain successful dominators of the night sky in the presence of passive and active jamming signals.

Chapter IV

Behavioral enhancement by visual cues during obstacle avoidance in echolocating bats

Chapter Notes

One of the prevailing misconceptions concerning bats is that they are blind or have no use for visual information. This is understandable given their mostly nocturnal lifestyle and relatively small eyes. However, studies have shown that bats are capable of using visual information for a variety of purposes including navigation and foraging. Indeed, some studies have gone so far as to suggest that visual cues dominate auditory ones in certain scenarios. It is unclear, however, what conditions influence the prioritization of information from some sensory systems over others.

In previous chapters, some of the limiting factors of echolocation are explored along with how bats might adapt their own vocalizations to compensate. The focus of this chapter is to attempt to quantify the behavioral responses of *Eptesicus fuscus* trained to navigate around an obstacle to obtain a food reward when the obstacle is composed of difference combinations of auditory and visual stimuli. This is a first step toward understanding the relative importance of visual and auditory sensory modalities during goal-oriented behaviors.

ABSTRACT

Studies have shown that bats are capable of using visual information for a variety of purposes, including navigation and foraging. Indeed, some studies have gone so far as to suggest that visual cues dominate auditory ones in certain scenarios. Additional inquiry is required to investigate the relative importance of visual and auditory sensory modalities when completing different tasks. A first step requires a characterization of how bats respond to different combinations of sensory cues. Here we quantify the behavioral responses of *Eptesicus fuscus* trained to navigate around an obstacle to obtain a food reward when the obstacle carries different combinations of auditory and visual cues. To do so, we utilize a new method that eliminates the confounds typically associated with testing bat vision and precludes auditory cues. We find that the presence of visual and auditory cues together enhances bats' avoidance response to obstacles compared to cues requiring either vision or audition alone. Analysis of flight and echolocation behaviors, such as speed and call rate, did not vary significantly under different sensory obstacle conditions, and thus are not informative indicators into how a bat responds to each obstacle stimulus type. These findings advance the understanding of the relative importance of visual and auditory sensory modalities in guiding obstacle avoidance behaviors.

INTRODUCTION

Animals navigating and foraging in their natural environments must not only detect biologically relevant signals, but also must determine how to use that sensory information for a given task. An animal's surroundings are generally filled with noise and ambiguous signals, and the information from multisensory input can contain more information than signals from any single sensory modality, or the signals carried through one sensory modality can contain more reliable information than that in others. Therefore, being able to combine stimulus information across multiple sensory modalities, and to subsequently weight these different sensory inputs, is crucial to disambiguating information about the world, forming unified perceptions of objects, and guiding probabilistic decision-making. For example, both male and female big-clawed snapping shrimp (*Alpheus heterochaelis*) use the same visual display in aggressive and mating interactions, requiring individuals to assess sex via chemical cues in order to respond to the display appropriately (Hughes 1996). Gray squirrels (*Sciurus carolinensis*) emit alarm signals among conspecifics which contain both a visual and auditory component and in populations inhabiting urban environments with more auditory noise, individuals rely more heavily on the visual component than their more rural counterparts (Partan *et al*. 2010).

Bats serve as an excellent model for studying multi-modal sensing and decision processes. Microchiroptera[8] are well known to use auditory information (via passive listening or by active biosonar) for prey capture and foraging (Bell 1982; Marimuthu and

[8] See the dissertation overview for comments on phylogenetic terminology.

Neuweiler 1987; Ryan 1987; Anderson and Racey 1991; Faure and Barclay 1994; Russo *et al.* 2007; Gomes *et al.* 2016), a prevailing misconception is that echolocating bats are blind or have no use for visual information (Thiagavel *et al.* 2018). This can be attributed to their mostly nocturnal lifestyle and relatively small eyes. Although many bat species rely extensively on biosonar for many facets of life, echolocation is only functional over relatively short distances, due to its reliance on high frequencies that attenuate rapidly in air (Lawrence and Simmons 1982; Jakobsen *et al.* 2013). Thus, bats also use vision for tasks such as navigation (Davis 1966; Williams *et al.* 1966; Layne 1967; Griffin 1970; Höller and Schmidt 1996), escape behaviors (Chase 1983; Mistry 1990), and predator surveillance (Eklöf 2003). Our goal is to document bat responses when multisensory cues in the auditory and visual domains are present.

Vision and hearing are closely coordinated senses. In many organisms, a major function of sound localization is to direct the eyes to the source of a sound (Heffner and Heffner 1992; Heffner *et al.* 1999). However, when visual cues and cues of other sensory modalities conflict, visual input often dominates (Wilcoxon *et al.* 1971; Bekoff 1972; Posner *et al.* 1976; Uetake and Kudo 1994; Witten and Knudsen 2005; Ward and Mehner 2010). Accordingly, we can find various instances in which animals have demonstrated visual dominance over other senses (Bekoff 1972; Posner *et al.* 1976; Uetake and Kudo 1994). Which, if any, sense dominates perception depends on the type of task being performed (Parker and Robinson 2017). Generally speaking, spatial navigation tasks, including those that require obstacle avoidance, tend to depend largely on vision (Welch and Warren 1980). This is an intuitive strategy for an organism whose primary

sense is vision, but not necessarily for species whose primary sense is audition, such as echolocating bats.

Prior studies of bats have suggested that task performance using echolocation may be enhanced by the presence of visual cues, especially in dim-light conditions like those found at dusk or dawn. *Eptesicus nilsonii* in southern Sweden may use visual cues during prey search to locate bright white moths that are active just above and within tall grass (Jensen *et al*. 2001; Eklöf *et al*. 2002). It has also been suggested that bats may exhibit visual dominance in some tasks, even when both auditory and visual cues are present and discriminable (Danilovich and Yovel 2019). For example, bats presented with hypothetical escapes via transparent, rigid windows through which light entered a darkened room or maze, tended to collide with these structures, countering the assumption that their echolocation should have alerted them to the obstacle (Davis and Barbour 1965; Chase 1981; Chase 1983; Mistry 1990). However, it has also been shown that bats presented with smooth vertical surfaces, such as glass windows, do not receive returning echoes until they are in very close proximity to the structure due to the angles at which sound is reflected (Greif *et al*. 2017), raising the possibility that bats did not receive echoes from the surfaces presented in the above named studies with enough time to abort their escape attempts.

Other studies suggest that vision has a deleterious effect on task performance when combined with echolocation. Free flying *Myotis lucifugus* made a greater number of collisions with a trailer when the exterior lights were on (McGuire and Fenton 2010). In situations where bats appeared to be guided by sight, they often improved their performance when the eyes were covered (Griffin and Galambos 1941). This may be

because these experiments were conducted in light conditions that were too bright and impaired visual function. It has been demonstrated that normal levels of room illumination (~377 lux; similar to lighting in an interior classroom) appear to impair obstacle avoidance in *M. lucifugus* and performance was best in very dim conditions (~1 lux; similar to a night with a full moon) (Bradbury and Nottebohm 1969).

The extent to which bats utilize visual cues when echolocation cues are available is an ongoing topic of inquiry. As a whole, the study of audiovisual integration in echolocating bats is incomplete and would benefit from the addition of more studies examining this phenomenon under a variety of conditions across many species. From the literature, it is clear that bats possess the capacity to see and that vision plays a role in their natural behaviors (Curtis 1952; Chase and Suthers 1969; Suthers *et al.* 1969) but which modality, echolocation or vision, is prioritized appears to depend on the environment, stimulus strength, species, and task.

To understand the relative importance of visual and auditory sensory modalities in obstacle avoidance tasks, we conducted a set of behavioral experiments in which we quantified navigation of the laryngeal echolocating big brown bat (*Eptesicus fuscus*) in response to visual and sonar obstacles that resulted in different combinations of audiovisual cues. One of the challenges that past studies have failed to overcome is establishing a method to present stimuli in the visual domain without providing information in the auditory domain, which is essential to determine which cues the bat is using. The present study exploited a novel method to display visual obstacles that yielded no echo returns. The goal of this study was to determine whether bats would rely solely on vision for performing obstacle avoidance and whether the presence of visual and

auditory cues would differentially affect the behavioral responses observed during task performance.

METHODS

Animals and Setup

We designed an obstacle avoidance task in which three wild-caught adult female big brown bats [*Eptesicus fuscus* (Palisot de Beuavois 1796)] were trained to fly into a box suspended from the ceiling (Fig. 4.1) for a food reward. Experiments took place in a large room (6 x 6 x 2.5 m) under IR illumination. Bats were captured in North Carolina under collecting permit 17-SC01070 issued by the North Carolina Wildlife Resources Commission and were housed and trained at Johns Hopkins University according to all procedures set forth by the Institutional Animal Care and Use Committee (protocol number: BA17A107). A food reward of mealworms (*Tenebrio molitor* larvae) was given when the animals entered the box (60 cm long x 70 cm wide x 65 cm tall), requiring them to navigate past the obstacle, if present, and land on any one of the three enclosing walls. In trials when bats did not enter the box, or they landed on the outside of the box, animals were not given rewards. A fan-operated mist-producing apparatus was used create a column of water vapor in front of the box opening during training and testing. The opening of the box remained unobstructed for control trials and was partially obstructed with an obstacle during test trials.

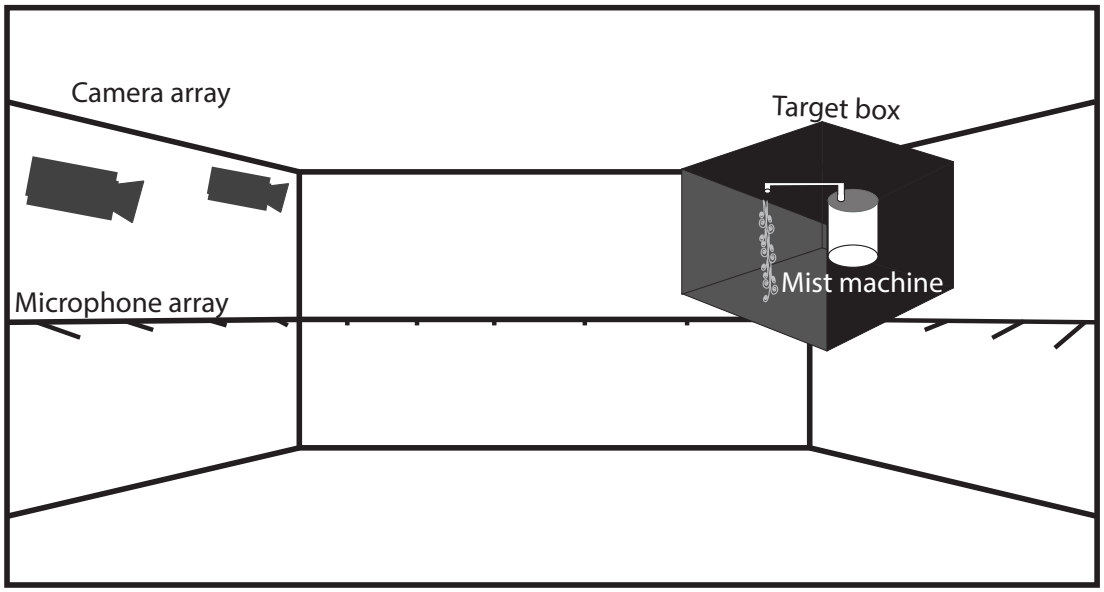

Figure 4.1. Schematic of the flight room. High-speed cameras positioned directly across from the box opening and a synchronized microphone array on all four walls allowed for the recording of bat position and echolocation throughout the room. Bats were released directly opposite of the target box and were rewarded upon entry.

Obstacles

We created obstacle conditions (Fig. 4.2) in which the three bats (Table 4.1) were presented with acoustic-only cues (A+V-, n = 43 trials), vision-only cues (A-V+, n = 38 trials), acoustic and vision cues (A+V+, n = 35 trials) or no obstacles (A-V-, n = 56 trials). A-V+ cues are challenging to create because this condition requires an acoustically transparent object that still has serves as a visual obstacle in the flight path. To create this condition, a laser was used, in conjunction with the column of mist. The mist supplied additional airborne particles to increase the scattering of light resulting in increased visibility of the entire laser projection. The result was a thin beam of solid green light (520 nm, 3 mm diameter), which according to electroretinograms, should be near the peak sensitivity of *E. fuscus* (Hope and Bhatnagar 1979) and easily detectable. *Eptesicus'* ability

to detect the 520 nm light was also verified in a separate behavioral experiment (see below). A+V- cues were constructed by placing thin (5 mm diameter), flexible pieces of rubber wrapped in a thin string of unlit LEDs in front of the opening. A+V+ were these same LED-wrapped rubber pieces with the lights turn on. To prevent bats from relying on spatial memory to avoid obstacles, each was randomly positioned in either a horizontal or vertical configuration and placed in either center or off-center locations at the box opening across trials. The entire box was covered in non-reflective black felt in order to minimize strong visual cues, even when partially illuminated by the light of the obstacles. The felt also served to attenuate echoes (Warnecke *et al.* 2018). Experiments were conducted under long wavelength ambient-light conditions to which *E. fuscus* is not sensitive (Hope and Bhatnagar 1979).

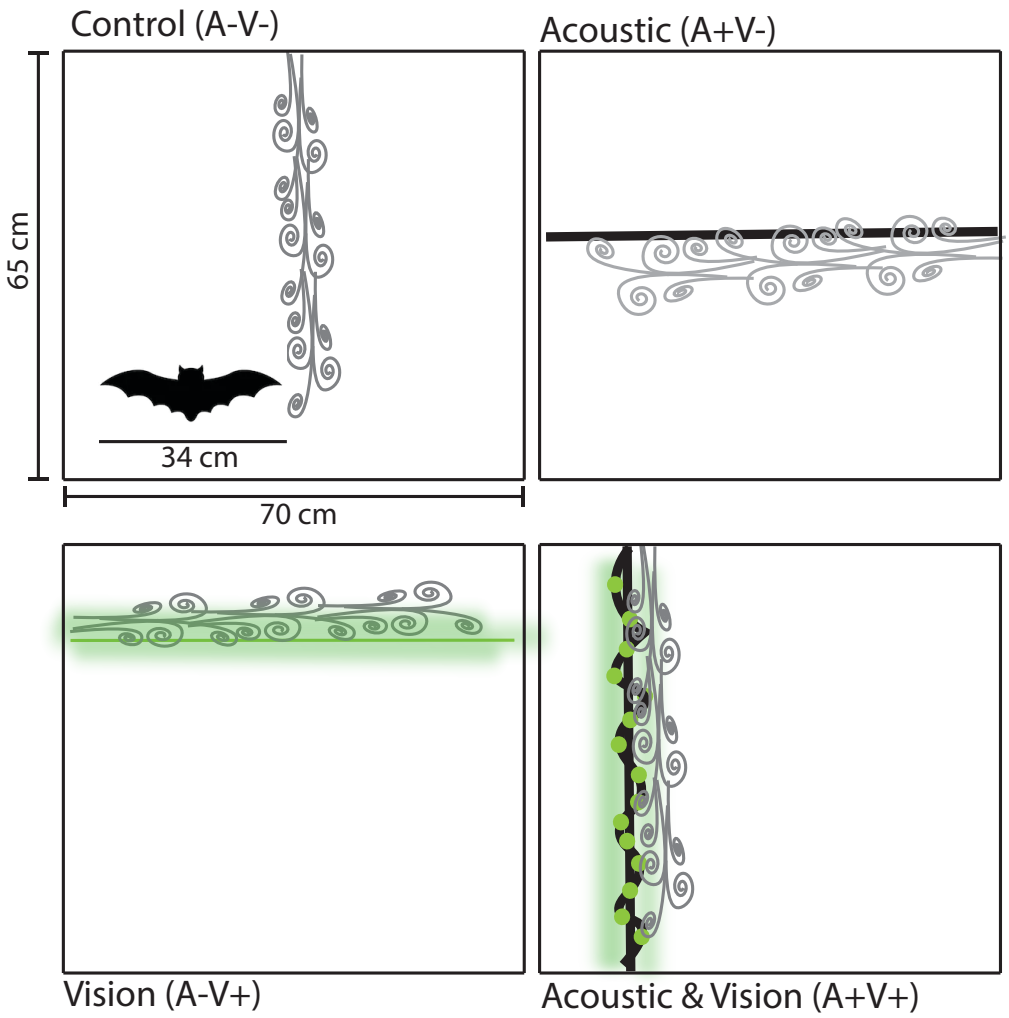

Figure 4.5. Four examples of the obstacles used in the experiment. Top left: the control condition had only mist (represented by gray swirls) present, shown in the vertical-center configuration. Top right: the acoustic-only cue (represented by a black bar) located in the horizontal-center configuration along with mist. Bottom left: vision-only cue of laser beam (represented by the thin green line; surrounding green represents the minor light diffusion into the surrounding mist) in the horizontal-top configuration. Bottom right: acoustic and vision cue together (represented by a black bar with green dots representing the LEDs wrapped around it) shown in the vertical-left position. These are just some of the conditions presented; all possible configurations of vertical/horizontal and left/center/right or top/center/bottom for each of the four conditions (A-V-, A+V-, A-V+, and A+V+) were included in the study for a total 24 unique combinations. Image scaling is approximate.

Table 4.1. Number of trials per bat

Bat	Number of trials
G20	33
G40	60
O90	45

Audio-video Recordings

Each trial was video recorded with two high-speed Phantom Miro cameras (Wayne, NJ, USA) sampling at 100 frames s^{-1} and the trajectories of the bat within the flight room were reconstructed using DLTdv5 digitizing software (Hedrick 2008). Echolocation calls emitted during the trials were recorded with a 24-channel wide-band ultrasound microphone array (Pettersson Elektronik, Uppsala, Sweden). The camera and microphone systems were synchronously recorded, triggered via a TTL pulse generated with custom hardware. The resulting reconstructed flight trajectories, extracted from the digitized center of mass of the bat, and audio recordings were further processed and analyzed using custom MATLAB (Natick, MA, USA) scripts to extract acoustic parameters of the fundamental harmonic of the bats' echolocation calls, presented in Table 4.2, and kinematic parameters of the bats' flight, presented in Table 4.3. These custom scripts were developed as part of this dissertation and are detailed in the appendix.

Table 4.2. Echolocation call parameters.

	Parameter	Definition
Spectral Parameters	Peak frequency (kHz)	Frequency (kHz) with the most energy in a call.
	Start frequency (kHz)	Frequency at beginning of each call -20dB below peak frequency.
	End frequency (kHz)	Frequency at end of each call -20dB below peak frequency.
	Bandwidth (kHz)	Range of frequencies covered in a sonar emission.
Temporal Parameters	Call interval (ms)	Time between successive call onsets and the reciprocal of call rate (Hz).
	Call duration (ms)	Duration of individual sonar emissions.
	Sweep rate (kHz/ms)	Quotient of bandwidth ÷ call duration; describes the slope of a frequency modulated call.
	Sonar Sound Groups	Clusters of echolocation calls with similar call intervals embedded in a sequence of calls with longer call intervals.

Table 4.3. Kinematic parameters.

Kinematic Parameter	Definition
Velocity (m s^{-1})	Average 3D velocity of bat trajectory
Turning Angle (°)	Maximum change in subsequent angles between the bat tangent and the vector between the bat and target box.

Statistical Analyses

All statistical analyses were conducted in R v. 3.6.3 (https://www.r-project.org) using the lme4 package (Bates *et al.* 2015) to generate linear mixed effects models (LMMs) or generalized linear mixed effects models (GLMMs). Models were analyzed with ANOVAs, as described in the results, with individual bat identities used as a random effect. For analyses of temporal and spectral acoustic parameters, the individual trial was also used as a random effect. Planned contrasts and post hoc analyses were carried out using the multcomp package (Hothorn *et al.* 2008), adjusting p-values using the Bonferroni-based false discovery rate method (α= 0.05).

Behavioral Determination of 520 nm Light Detection

To confirm that the light generated by the laser was detected by the bats, two additional *E. fuscus* were trained in a separate set of behavioral experiments using a 2-alternate forced choice (2AFC) setup. In this paradigm, bats were trained to crawl to the arm of a Y-platform in response to the laser stimulus projected onto a piece of cloth (Fig. 4.3). The laser 520 nm beam was manually oriented to either the left or right in alignment with the arms of the platform. They were rewarded with a mealworm for crawling towards

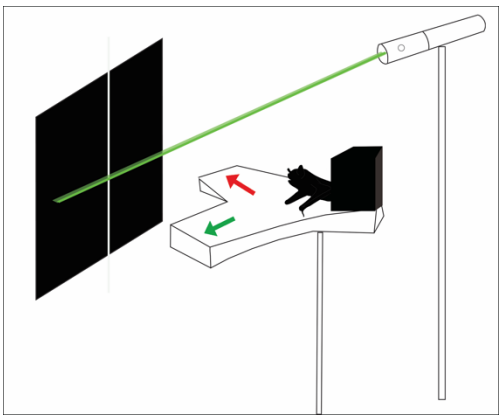

Figure 4.3. Diagram of the Y-platform in the 2AFC task. Bats were presented with a light stimulus generated by the same laser used in the obstacle avoidance portion of the experiment. The light was projected onto a piece of black felt in front of the Y-platform and were required to choose the arm corresponding to the side of the felt onto which the laser was projected. Bats were rewarded with mealworms for correct choices. In the above example, the bat should choose the left arm (green arrow).

the side where the laser was projected. One individual was tested on 75 trials, and the other on 100 trials; a permutation test was used to estimate the expected percentage of correct responses to determine if the animals performed significantly above 50% in the visual detection task. This generates a cutoff percentage, or performance threshold, for ensuring bats reliably detect the presence of the signal (i.e. the laser) at a rate that is statistically better than chance.

RESULTS

Behavioral Detection of 520 nm light

In the two-choice laser detection task, the two bats went to the correct arm of the platform in 87% (Bat 1) and 95% (Bat 2) of trials, which is above their respective chance performance of 58% and 60% (Fig. 4.4). This confirms that the laser stimulus is indeed detectable by *E. fuscus.*

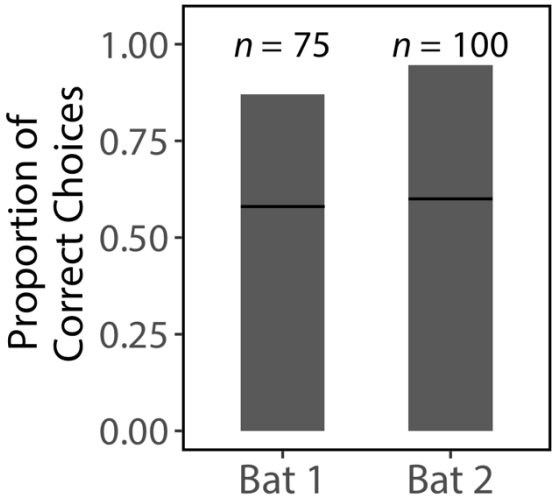

Figure 4.4. Results of the 2AFC task. Proportion of correct choices on the Y-platform for two individual bats. The black line depicts each bat's performance threshold based on the number of trials they completed (n) and an alpha level at 0.05. Surpassing this threshold indicates that bats were performing significantly above chance. Both individuals' performance demonstrated that 520 nm laser stimulus was above detection threshold.

Obstacle Avoidance Performance

In the flight experiment, bats were required to use echo acoustic and/or visual cues to steer around an obstacle placed at the opening of a box to receive a food reward.

Performance was compared across four sensory conditions: A-V- (control), A+V-, A-V+, and A+V+. Obstacles were thin enough (≤ 5 mm) and the overall width of the box was large enough (70 cm wide) to accommodate the full wingspan of the individual bats (<35 cm) on at least one side of the obstacle. A chi-square test of proportions revealed that there was no significant effect of obstacle orientation (X^2 = , df = 1, n = 118, p = 1) or position (X^2 = 5.06, df = 4, n = 118, p = 0.28) on bat entrances to the box across sensory conditions, so we excluded these terms from subsequent models, grouping all trials using each obstacle stimulus type. There was a significant difference in the number of trials when the bats flew into the box across each stimulus type (GLMM with binomial error distribution, $F_{(3,167.21)}$ = 9.86, p < 0.001; Fig. 4.5). Bats almost always entered the box under unobstructed control conditions (98%). The percentage of flights into the box was

significantly reduced in A-V+ and A+V- conditions (84% and 83%, respectively) and even more so in A+V+ conditions (57%).

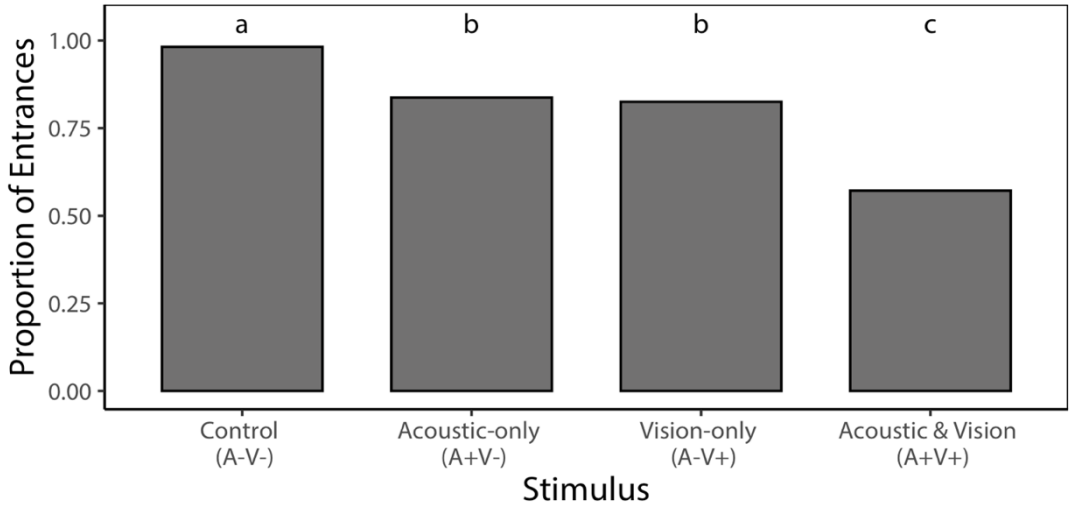

Figure 4.5. Proportion of entrances for each obstacle type. A+V- and A-V+ stimuli resulted in a significant decrease in the proportion of entrances and an even further decrease is observed with the A+V+ obstacles. Bars sharing a letter are not significantly different from each other.

We also determined whether bats contacted one type of obstacle more than another type. "Contacts" were defined as either colliding with the obstacle or touching it with a wing as it was passed. For 60% of A-V+ trials in which the bat entered the box, bats 'made contact' with the laser beam without attempting to avoid it or the mist column onto which it was projected, as indicated by the lack of observable changes in the flight trajectory. This is significantly more than the A+V- trials, in which contact was made with the echo-acoustic obstacle in 3% of trials (GLMM with binomial error distribution, $F_{(2,85.29)} = 36.12$, $p < 0.001$) There was no significant difference between A+V- and A+V+, in which no contacts were made in any trial.

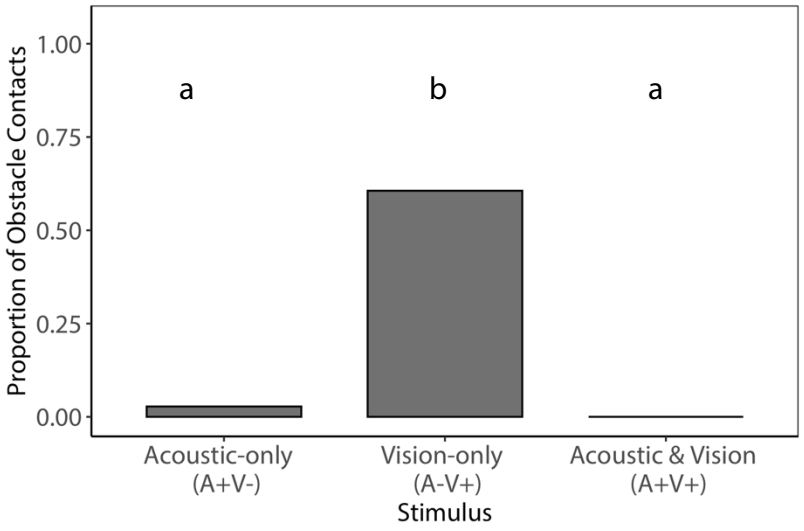

Figure 4.6. Proportion of contacts with obstacles. Bats make contact with the A-V+ obstacle approximately 60% of the time, significantly more than either of the other obstacle conditions.

Flight Analysis

3D reconstructions of each of the bats' flight trajectories (Fig. 4.7) were used to determine the animal's position during each recorded frame. Using this information, we calculated the average speed of the bat during each trial and compared this across each obstacle condition and whether bats entered the box (LMM). There was no significant difference in speed across obstacle conditions ($F_{(3,163.04)} = 0.46$, $p > 0.05$), but bats did fly significantly faster when they entered the box ($F_{(1, 163.65)} = 31.45$, $p < 0.01$) than when they avoided the box (Fig. 4.8). There was no interaction effect between stimulus conditions and the outcome.

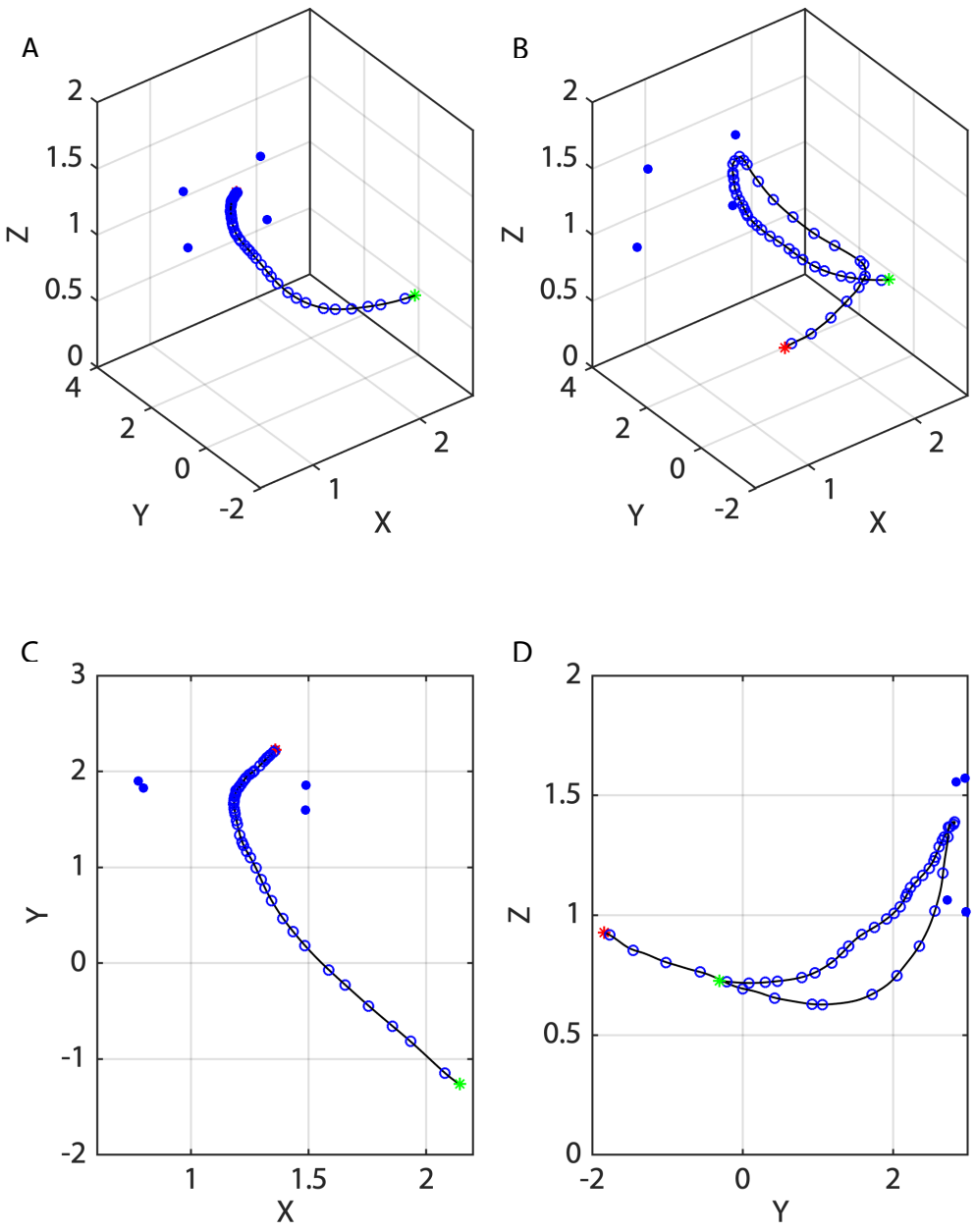

Figure 4.7. Example trajectories of a bat entering and avoiding the box. 3D views of an A) entrance and B) avoidance trial. The same trials are shown from C) top-down and D) lateral views. The black line represents a smoothed bat trajectory while each hollow blue circle represents the onset of a single echolocation call emitted by the bat. The start and end of the trajectory are indicated by green and red asterisks, respectively. The corners of the box opening are represented by the filled blue dots and are offset slightly in panels C and D for visibility.

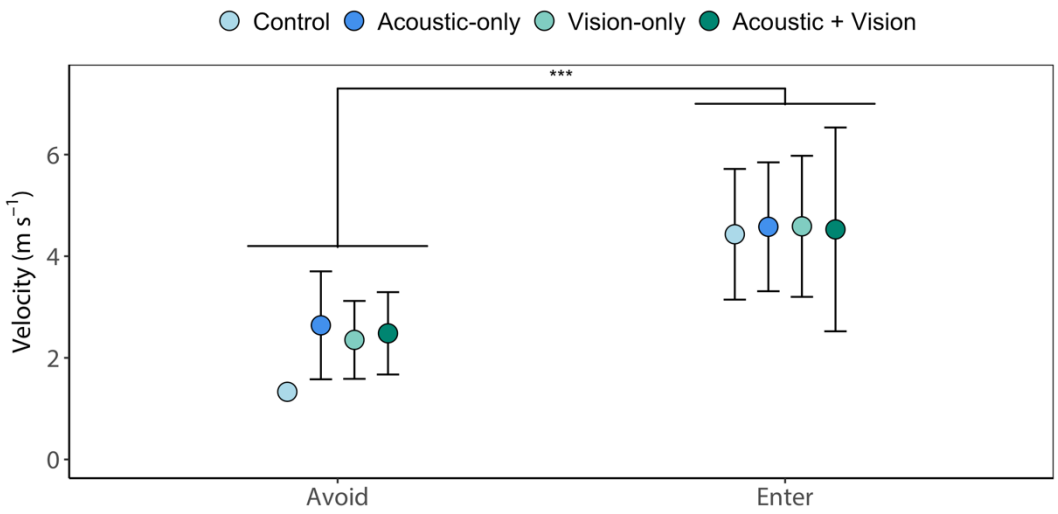

Figure 4.8. Velocity with respect to outcome. Velocity (m s^{-1}) of bats flying under each obstacle condition, separated by whether the bat avoided or entered the box. Bats fly significantly faster when they are entering the box compared to when they avoid it. There was only one trial in which a bat did not enter the box under control conditions, so only one values is displayed. Significance levels are as follows: $p < 0.001$ '***'; $0.001 < p < 0.01$ '**'; $0.01 < p < 0.05$ '*'; $p > 0.05$ 'NS'

Additionally, we decomposed the trajectories into 1.0 m bins to look at speed on a finer scale. These distance bins were compared across each obstacle type (LMM). We observe a significant interaction between the main effects of distance bin and obstacle type ($F_{(19,17433)} = 8.80$, $p < 0.01$), and planned contrasts of each obstacle type within each distance bin revealed significant differences primarily when the bat was within 0-1 m and 1-2 m of the box opening (Fig 4.9).

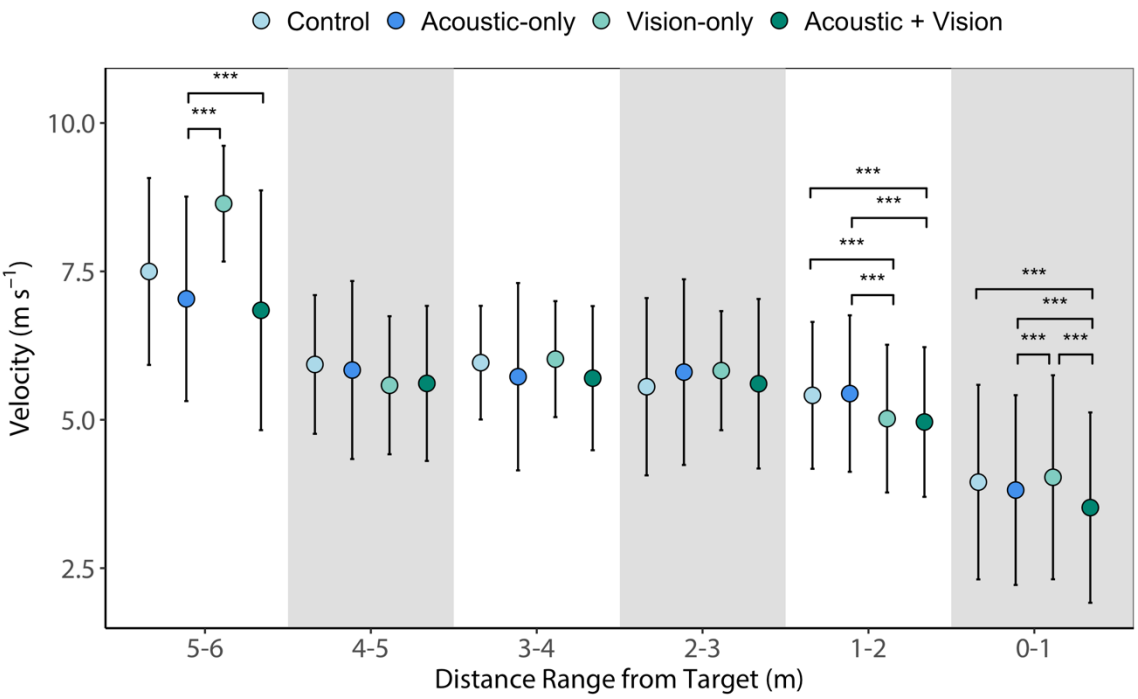

Figure 4.9. Velocity with respect to distance and obstacle type. We observe that most significant changes in speed occur when the bat is within 2 meters of the box opening. Circles represent the mean and error bars are the standard deviation. Note that the axes are reversed, with distance decreasing from left to right in 1 meter increments. Significance levels are as follows: $p < 0.001$ '***'; $0.001 < p < 0.01$ '**'; $0.01 < p < 0.05$ '*'; $p > 0.05$ 'NS'

Angle of Avoidance

We also analyzed the angle of avoidance in trials where the bat did not enter the target box. The angle of avoidance is defined as the maximum angle between the bat-target vector and the bat tangent when the bat is initiating its avoidance turn, which occurs at or before the minimum distance between the bat and the opening of the box. This measure can be used as a proxy for when the bat makes the decision to not enter the box. There was no significant difference (LMM, two-way ANOVA) in angle of avoidance across obstacle types ($F_{(3,22.58)} = 0.36$, $p > 0.05$), there was no significant difference in the

distances at which the turn occurred ($F_{(122.99)} = 0.0003$, $p > 0.05$), and we observed no interaction effects.

Echolocation Calls

We determined the position of the bat along each flight trajectory at the time of each echolocation call emission. The trajectories were then binned into 0.5 m increments relative to the position of the center of the box opening and we analyzed the number of calls produced in each distance bin and under each stimulus condition (negative binomial regression). As expected, bats increase the number of calls as distance to the box decreased and we observe a significant interaction effect between distance bin and obstacle type ($F_{(117,6718)} = 2.9$, $p < 0.01$; Fig. 4.10). There was also a significant difference between the number of calls emitted and whether or not bats entered the box ($F_{(1,259.32)} =$

2.68, $p > 0.05$). On average, bats emitted two more calls when they entered the box than when they did not.

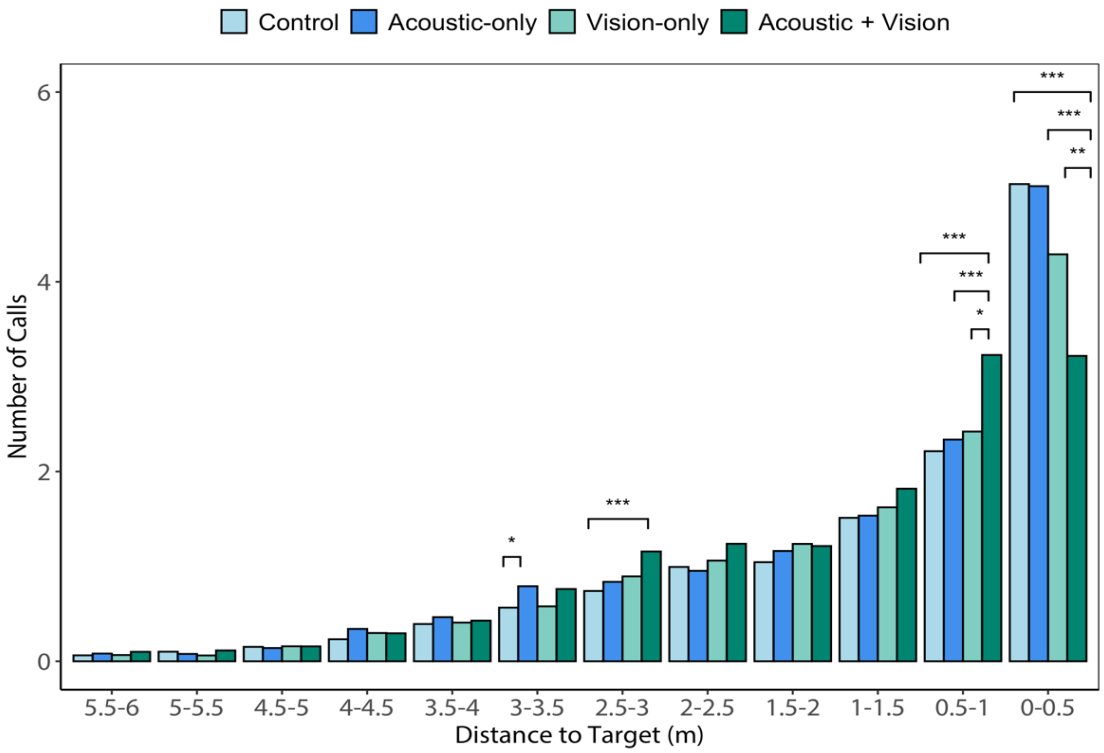

Figure 4.10. Number of calls with respect to distance and obstacle type. When bats are 0-0.5 meters from the box, they call significantly less in the A+V+ condition than in all other conditions, including controls. When the bats are 0.5-1 meter from the box, bats call significantly more in A+V+ condition than in the other conditions. Note that the axes are reversed, with distance decreasing from left to right in 0.5 meter increments. Significance levels are as follows: $p < 0.001$ '***'; $0.001 < p < 0.01$ '**'; $0.01 < p < 0.05$ '*'; $p > 0.05$ 'NS'

In many trials, audio and video recordings were captured in which the bat was not yet in flight and/or initially out of view in one or both cameras, making it impossible to re-create the trajectory at those points. These calls were not included in the previous analysis and instead were analyzed separately (negative binomial regression) to determine if the bats were calling more frequently at the beginnings of trials with different obstacle conditions or if the calls could be used to predict whether bats would enter the box. There

was no significant effect associated with the outcome of whether bats entered the box or not ($F_{(1,556.27)} = 2.18$, $p > 0.05$). There was a significant effect of the stimulus presented ($F_{(3,557.47)} = 2.18$, $p < 0.05$), but these effects were not statistically significant ($p < 0.05$) during post-hoc comparisons after adjusting for multiple comparisons. No interaction effect was observed.

Lastly, we analyzed several spectrotemporal acoustic parameters of bat sonar calls during each trial (see Table 4.4 for summaries of statistical results). There was no significant overall effect of obstacle stimulus type on spectral or temporal parameters. Since pulse interval is known to vary significantly with object distance, an analysis of pulse interval across 1 m distance bins was conducted with respect to the different obstacle conditions (LMM). The data show that there is a trending decrease in pulse interval as bats approach the box and that there is a significant interaction effect between distance bin and obstacle type ($F_{(15,5434)} = 2.54$, $p < 0.01$; Fig. 4.11). *Post-hoc* comparisons only result in a single significant comparison at 1-2 m between A+V- and A+V+ obstacles.

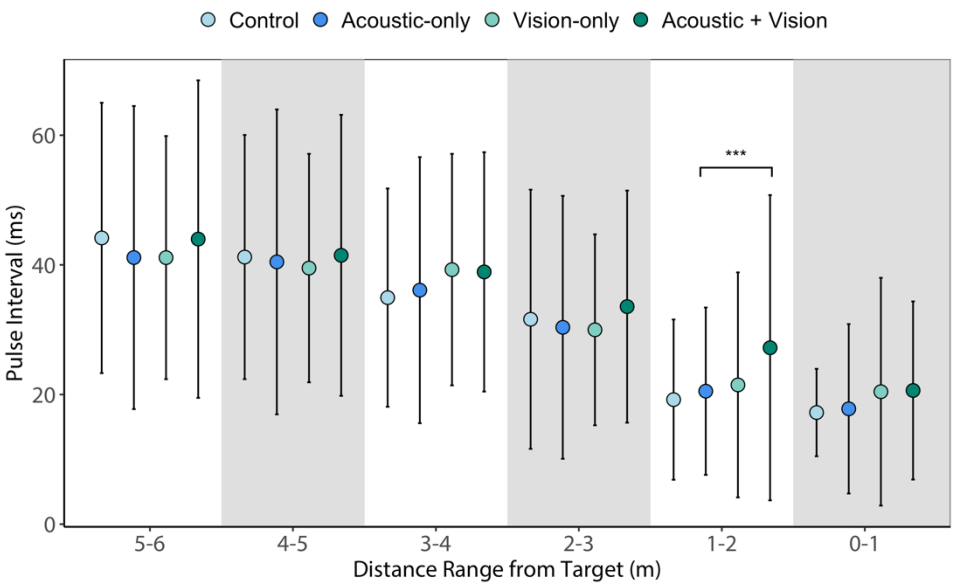

Figure 4.11. Pulse interval with respect to distance and obstacle type. While we observe a stereotypical decrease in pulse interval as the bat moves closer to the box, there is only a single significant interaction at 1-2 meters in which bats significantly increase the pulse interval between calls in the acoustic-visual (A+V+) condition compared to the control condition (A-V-). Circles represent the mean and error bars are the standard deviation. Significance levels are as follows: $p < 0.001$ '***'; $0.001 < p < 0.01$ '**'; $0.01 < p < 0.05$ '*'; $p > 0.05$ 'NS'

Additional significant differences in sonar sound groups, peak and start frequencies, and bandwidth are related to whether the bat entered the box or not. We observe an increase of approximately 124 Hz in start frequency in trials where bats do not enter the box. Peak frequency decreases by approximately 647 Hz and total bandwidth of calls decreases by approximately 300 Hz when bats do not enter the box. On average, bats produced three more sonar sound groups and increased their pulse interval by approximately 21 ms when they did not enter the box.

Table 4.4. Table of all statistical analyses conducted on temporal acoustic parameters and their corresponding contrast results.

	Parameter	Variables	*F*	Numerator degrees of freedom	Denominator degrees of freedom	*p*-value	Post-hoc comparisons
Temporal Features	Call duration	Stimulus	0.27	3	155.41	0.85	
		Outcome	3.12	1	149.15	0.080	
	Call pulse interval	Stimulus	2.57	3	145.79	0.56	
		Outcome	176.38	1	131.52	<0.001	***
	Sweep Rate	Stimulus	0.57	3	165.91	0.19	
		Outcome	1.75	1	160.71	0.64	
	Sonar sound groups	Stimulus	1.24	3	162.75	0.30	
		Outcome	35.21	1	163.11	<0.001	***
Spectral Features	Peak Frequency	Stimulus	0.70	3	161.94	0.55	
		Outcome	22.47	1	157.51	<0.001	***
	Start Frequency	Stimulus	3.14	3	173.89	0.027	A-V- / A+V- 0.904 A-V- / A-V+ 0.904 A-V- / A+V+ 0.589 A+V- / A-V+ 0.904 A+V- / A+V+ 0.060 A-V+ / A+V+ 0.050
		Outcome	9.08	1	175.09	0.0030	**
	End Frequency	Stimulus	1.57	3	159.18	0.20	
		Outcome	0.12	1	158.52	0.74	
	Bandwidth	Stimulus	2.21	3	180.18	0.089	
		Outcome	7.50	1	179.83	0.0068	**

DISCUSSION

Bats can use both visual and acoustic cues to navigate their environments. In some instances, such as short-range navigation under crepuscular light conditions, visual and acoustic cues may provide complementary information. However, there are frequently scenarios in which vision and echolocation do not provide complementary information. For example, when navigating long distances or detecting large objects at distances exceeding ~100 meters, echolocation no longer provides reliable information due to the high degree of atmospheric attenuation that limits the functional range of high-frequency calling (Lawrence and Simmons 1982; Holderied and von Helversen 2003; Stilz and Schnitzler 2012). Thus, vision would likely provide reliable cues for identifying landmarks or large obstacles. And in complete or near complete darkness, where visual cues are virtually absent, echolocation provides information about the location, size, texture, and motion of objects around which the bat maneuvers (Fenton *et al*. 2016). The question of how vision and echolocation interact arises in situations where both cues are available and provide useful information.

We employed a navigational paradigm to investigate the effects of multimodal cueing on obstacle avoidance in the laryngeal echolocating bat, *Eptesicus fuscus*. Specifically, we focused on the behavioral responses to stimuli that yielded cues within the visual and/or auditory domains, as these two senses are most often utilized in tandem and are both functional in distal sensing. We sought to determine if bats demonstrate either auditory or visual dominance in the context of spatial navigation and obstacle

avoidance. In nature, this might arise when dim-to-intermediate light levels could facilitate bimodal sensing.

When presented with a task that required entering a box that was partially obstructed by an obstacle, *E. fuscus* demonstrated behavior patterns that depended on the stimulus dimensions of the obstacle. Stimuli were constructed to provide echoic feedback, visual feedback, or both and bats' performance was analyzed across several echolocation and flight kinematic parameters. When visual and echo acoustic stimuli were presented simultaneously, the two cues (A+V+ condition) were combined into a multimodal composite signal (MCS). MCSs can result in several potential outcomes that depend firstly on whether the individual component signals convey the same information (i.e. they elicit the same behavioral response) and secondly on how the conveyed information of the combined signal influences behavior (Partan and Marler 1999). If the two component signals elicit the same behavioral response, then the two are said to convey redundant information while different behavioral responses are the result of non-redundant information (Fig. 4.12).

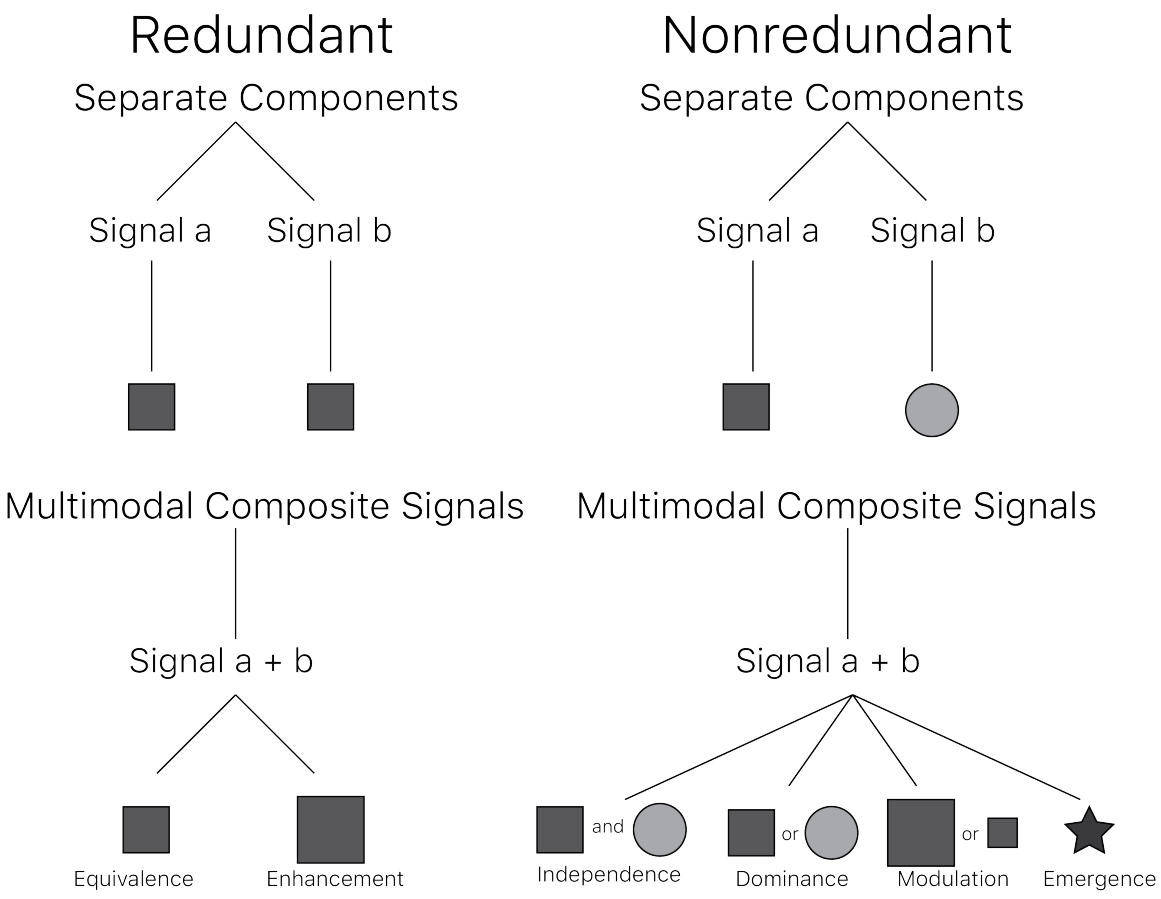

Figure 4.12. Multimodal composite signal classification. The diagram differentiates redundant and nonredundant multimodal composite signals (MCSs). Signals a and b represent a single cue operating within each sensory modality (i.e. acoustic and visual). Each shape represents a unique response type and the same shape of a different size represents an increase (larger size) or decrease (smaller size) in the intensity of the response. The category into which each type of MCS falls, based on its associated response is listed below the shape combinations. Definitions are based on those provided by Partan and Marler (1999).

We anticipated that MCSs, consisting of visual and auditory stimuli, in the obstacle avoidance task would offer redundant information and that when presented together, would result in an equivalent or enhanced response (i.e. percentage of trials in which the bat entered the box). This is because redundancy is one of the simplest ways to counteract a noisy environment or discriminate potentially ambiguous signals by having multiple sensory modalities supply 'backup' information. Although our laboratory-based task did not introduce noise, environments frequented by bats in the wild often contain

extraneous sounds, such as signals produced by nearby conspecifics or reverberant echoes from highly cluttered environments (Dusenbery 1992; Schnitzler and Kalko 2001). The results of our study suggest that visual and auditory cues provide redundant information to the bats performing in the obstacle avoidance task reported here. Specifically, we observed that visual cues alone (A-V+) and acoustic cues alone (A+V-) resulted in a similar reduction of successful entry to the box when compared to the unobstructed control condition. When combined into MCSs (A+V+), the bats showed an even further decrease in entry to the box. This suggests that our multimodal signal results in an enhancement effect. Similar effects have been observed in the eastern grey squirrel (*Sciurus carolinensis*) which displays enhanced responses to multisensory, audio/visual components of a conspecific alarm signal compared to either unisensory component (Partan *et al*. 2009).

The position and the orientation of the obstacle had no impact on whether bats entered the box. When bats did enter the box, they rarely made contact with physical objects, and thus avoided potential physical discomfort associated with a collision in A+V- and A+V+ conditions. When navigating in proximity to the A-V+ obstacles, bats frequently flew through them breaking the beam of the laser with their wings and occasionally, the entire body. In psychophysical experiments that probe the detection, discrimination, and scaling of physical stimuli (Munoz and Blumstein 2012) behavioral responses are used to make inferences about perception (Shettleworth 2009). While we demonstrated that the laser stimulus was detectable by *E. fuscus* and that our obstacle conditions generated different behavioral responses, we do not yet have data to make inferences about the bat's perception of the A-V+ obstacle.

It appeared that bats did not treat the laser beam as a solid object, based on their high percentage of 'contact' with the obstacle, but their decreased number of entrances suggest that they did treat the laser beam as either an obstacle or other aversive stimulus. Since the laser obstacle yielded no tactile feedback when it was contacted by the bat in the same way solid echo-acoustic and visual/echo-acoustic obstacles provided, bats appear less likely to attempt to avoid 'collisions.' In the future, this could potentially be addressed by an experimental test on a 2D plane, with bats crawling to navigate around obstacles and administering an aversive stimulus, such as a mild shock similar to those given in rodent studies, whenever the bats made contact with the beam. This could potentially result in one of three outcomes: 1) no change in behavior – bats continue to make contact with the obstacle with no change in percentage of avoidance; 2) the obstacle is treated the same as the echo-acoustic obstacle and no contact is made with the beam and there is no change in the percentage of avoidances; 3) the obstacle is treated the same as the visual-acoustic obstacle and bats further increase their percentage of avoidances. This approach would also have the drawback of requiring additional training that may make it difficult to separate learned avoidance behaviors from innate object responses, altering the scope of the experiment. Moreover, the effects of punishment on bat behavior are not well documented, as such techniques are rarely employed.

Often, we analyze echolocation and flight parameters as indicators of the information bats are gathering about their environments. In this experimental setup, bats flew faster when entering the box. When analyzing speed on a finer scale we observe an interaction effect between distance to the box opening and stimulus obstacle condition and the speed at which the bat is traveling. When the bat is close to the opening of the box

(1-2 meters), we see significant decreases in speed when bats are presented with the visual-only and acoustic-visual obstacles, compared to the control and the acoustic-only conditions. At 0-1 meters, we observe a significant decrease in speed when bats are presented with the acoustic-visual obstacle compared with all other conditions. This suggests that bats wait until they are very close to the potential obstacles before altering their flight speed.

Some acoustic parameters of the bats' echolocation calls showed similar distance-dependent relationships with the obstacle type being presented. The pulse interval significantly increased when navigating around the acoustic-visual obstacle compared to the acoustic-only obstacle at 1-2 meters. We also note the significant increase in the total number of calls between 0.5-1 meters when presented with the acoustic-visual obstacle compared to all other conditions, followed by a significant decrease at 0-0.5 meters. Overall, bats tended to emit slightly more total echolocation calls when they entered the box than when they did not.

While the documented changes in flight and echolocation can be difficult to interpret in terms of the bats' perception of the obstacles, we can conclude that the addition of visual information to the active sensing of echolocation has the effect of altering the way individual bats choose to interact with their environment. This is consistent with the recent study conducted by McGowan and Kloepper (2020) in which wild *Tadarida brasiliensis* are documented exhibiting different echolocation patterns when flying during the day compared to night. Future iterations of this experiment should introduce new behavioral paradigms to further test which environmental contexts influence multimodal sensory processing. Neurophysiological experiments may also

contribute to our understanding of multimodal sensing by characterizing the underlying neural processes that mediate responses to different combinations of visual and acoustic stimuli.

Chapter V

Quantification of flight behaviors of *Hipposideros alongensis* in open and cluttered environments

CHAPTER NOTES

This work was conducted as part of a field course on animal communication on the Vietnamese island of Cát Bà in collaboration with the lab of Vu Dinh Thong. Drs. Annette Denzinger and Hans-Ulrich Schnitzler obtained funding for this excursion through the University of Tübingen, Germany's Reinhard Frank-Stiftung. Travel awards from the Department of Psychological and Brain Sciences at Johns Hopkins University covered airfare and equipment expenses. In addition to providing research experience, the goal of this course was to also foster positive relationships among international scientists. In addition to groups from Vietnam and Germany, we were joined by the lab of Yossi Yovel from Tel Aviv University, Israel. This study is a snapshot of the data collected for a much larger study aimed at identifying how species-specific differences in wing morphology and echolocation repertoire contribute to observable changes in flight behaviors either through intrinsic constraints (morphology and limits of echolocation

range) or active modification of parameters (flexible spectrotemporal features of echolocation).

This experiment was partially conducted to document the kinematic parameters of flight for species of bat for which this information does not exist. We were also inspired to confirm some of the claims made in a paper published by Norberg and Rayner (1987), reporting the most extensive correlation of flight speed with linear wing measurements across species. Though some measurements are outdated or cannot be confirmed with reproducible datasets, many of the assumptions follow those of aerodynamic theory and represent an intellectual jumping off point for exploring flight kinematics and its relationship to behavior and ecological constraints. The ultimate goal will be to create a compendium of flight behaviors that includes detailed measurements of bats engaging in a variety of activities, such as free-flight, prey capture or food competition, and obstacle avoidance and to relate results of these studies to both to echolocation behaviors and detailed geometric morphometric analysis of bat wing characteristics.

In addition to the geometric morphometrics, the extended analysis of this dataset will include the characterization of synchronized echolocation and flight behaviors during navigation in an open or cluttered novel environment to determine how the presence of obstacles results in mid-flight adaptation to the environment. While it would be informative to determine how these changes occur across individuals, since we know that individuals vary in their flight and echolocation already, it was not feasible to attempt this in the field. Part of the challenge for the bats is that they are navigating in a completely novel environment (a mesh tent) and allowing them to fly in both conditions could potentially lead to a bias in whichever condition came second. Large enough sample sizes

could help identify this effect or more resources to increase the amount of change in each condition could simulate novelty in the second condition. But with limited time, resources, and number of bats collected, we decided to leave this aspect of the experiment for possible future studies.

ABSTRACT

For many animals, efficient navigation of natural environments requires significant energy investments in self-propelled locomotion. Bats are the only mammal capable of true, powered flight and depend on it to forage for food, find mates and suitable roost sites, and perform complex tasks such as obstacle avoidance. Echolocating bats are a powerful model for examining how an active sensory process, such as echolocation, allows for the acquisition of information about its surroundings and the subsequent modification of flight behaviors. Although all bats need to orient in three dimensions, different species have evolved different flight strategies that are strongly influenced by their preferred foraging habitats. Here we provide the first known kinematic description of the flight behaviors of the bat *Hipposideros alongensis*, which inhabits cluttered forest environments. We quantify reconstructed 3-dimensional flight trajectories of wild adults in novel open or cluttered environments to document specific flight parameters such as velocity and maneuverability. This ongoing work will provide insight into how this species has developed strategies to optimize flight for their specific ecological needs in complex foraging environments and update existing knowledge in the field.

Introduction

There are currently over 1,300 documented species of bat occupying ecological niches in every part of the world excluding the polar regions and some islands (Nowak 1994; N.B. Simmons 2005). Across this extremely varied order, individual species of bat must cope with different types of problems, given their habitat and foraging preferences. For example, bats hunting in dense forests for quick moving prey face different challenges than nectivorous bats that travel long distances to locate stationary food sources. Studies that aim to describe these challenges and how bats might develop species-specific strategies for overcoming them often focus exclusively on echolocation, resulting in a dearth of studies documenting how bats might modify flight behavior in various conditions, especially those that replicate natural environments.

Analyses of echolocation dominate field studies most likely because acoustic data is generally easier to collect than kinematic data. Acoustic recordings can be made from a distance with a relatively lightweight equipment setup and does not require many additional components such as lighting. Kinematic measurements often require the capture of the bat, or at minimum luring the bat into the volume of space observed by the recording equipment (Corcoran and Conner 2014; Jones *et al*. 2018). Some measurements can be made with onboard sensors temporarily affixed to a bat large enough to carry them (Cvikel, Levin, *et al*. 2015), but still requires initial handling of the animal. Bats can then be released into the environment and data retrieved later from the logging device. This unfortunately, gives a limited view of the types of environmental elements the bat is navigating around. Though it requires extensive equipment setup and

post-processing of data, 3D motion capture systems have the potential to provide for a more comprehensive descriptor how the bat interacts with its physical environment.

Obstacles, such as dense forest foliage, not only generate acoustic clutter that can make echolocation difficult, it also creates physical clutter that requires the bat to perform rapid maneuvers, often at high speeds that are achieved during prey pursuit. For this successful navigation of complex environments to occur, bats must coordinate sensory input (via echolocation, vision, or somatosensation) and motor output. This has been demonstrated in what is one of the most commonly used species for scientific study, *Eptesicus fuscus*, an aerial insectivore that emits frequency-modulated (FM) echolocation calls. Falk *et al*. (2014) used high-speed video and audio recordings to reconstruct 3D flight trajectories, sonar beam aim, and acoustic emission patterns as bats captured prey in either open or cluttered environments. Bats were found to adapt their call structure, temporal patterning, and flight speed in response to changes in their environment. This suggests a dynamic shift in flight and vocal strategy relative to the complexity of the environment. Because bats demonstrate species-specific echolocation repertoires that are often adapted to specific contexts (Schnitzler and Kalko 2001; Surlykke *et al*. 2014), it would not be unexpected that bats might also exhibit species-specific flight parameters that are similarly modified to meet environmental demands.

This was partially addressed in 1987, when Norberg and Rayner published an ecological outline of wing morphology and its relationship to flight in bats. They explored flight performance and demonstrate that mechanical constraints underpin the relationship between flight morphology, flight behavior, and ecological niche. This work provides a broad overview of how species-level differences in the relationship between

simple wing measurements and flight speed and represents the most complete list of wing morphological data to date. Included in the analysis are data or partial data from 257 species spanning 16 families on mass, wingspan, and wing area – the most common linear measurements[9] used to describe wing morphology.

In an effort to expand upon this existing data in how flight kinematics might change in different environmental conditions, I utilize a subset of 3D data flight to provide a detailed account of flight behaviors in an understudied species from southeast Asia. The Ha Long leaf-nosed bat [*Hipposideros alongensis* (Bourret 1942)] is endemic to Vietnam and has only been identified as a distinct species within the last decade, with two subspecies *H. a. alongensis* and *H. a. sungi* which reside on Cát Bà Island and mainland Vietnam, respectively (Thong *et al*. 2012).

H. alongensis shares similar echolocation repertoires as other Hipposiderid and Rhinolophid bats in that they produce multi-harmonic signals composed of a long constant frequency (CF) component terminated by a short downward frequency-modulated (FM) component. Most energy is contained in the second harmonic (Pye 1980; Heller and Helversen 1989; Schnitzler and Denzinger 2011) and the frequency of the CF component is species-specific with little variability between individuals.

[9] Linear measurements are exactly that – linear – and struggle to fully explain many biological shapes because they do not adequately capture curvatures. Geometric morphometrics, such as those proposed in the chapter notes, can capture the variation along the entire outline of the shape in question, creating a multivariate descriptor for more accurate comparison, so long as homologous structures can be identified across all samples. This, in combination with traditional linear measurements, has the potential to describe and predict important ecological aspects of comparative flight performance in bats, given a large enough sample of directly comparable data.

Echolocation, along with overall size and facial and ear features, are reliable ways to characterize this species in the field.

At the time of writing, published knowledge on this this species is restricted primarily to taxonomy and conservation status (Puechmaille *et al*. 2009; Thong *et al*. 2012; Kruskop 2015; Nga and Tung 2018). This study represents a unique opportunity to document flight behaviors for the first time in this vulnerable and declining species and to quantify the kinematic parameters of a species that regularly navigates and forages in dense tropical forests.

MATERIALS & METHODS

Animals

Wild *Hipposideros a. alongensis* were captured using harp traps outside of Trung Trang Cave within the Cát Bà National Park on Cát Bà Island, Hải Phòng, Vietnam. Bats were captured under the supervision of Vu Dinh Thong, a collaborating investigator from Vietnam University of Science and Technology. Individuals were kept in separate cloth bags and temporarily stored near the study site. Prior to placement into the experiment, we examined each bat, took a photograph, and noted basic information on age, sex, forearm length, and general health. The hair on the top of the head was trimmed with scissors to prevent using potentially re-captured bats on subsequent nights. Data for this experiment[10] were collected from 12 *H. a. alongensis* (4 females, 8 males). Bats were all adults or sub-adults, determined by the epiphyseal gap between the metacarpal and proximal phalanx (Brunet-Rossinni and Wilkinson 2008).

Experimental Setup

Trials included in this study were conducted for five nights in mid-August, 2019 and took place inside of a mesh tent (4 m long, 4 m wide, 3 m tall; Fig. 5.1) that was erected at the study site each night. This site is approximately 150 m from the entrance to the cave, adjacent to the Trung Trang Cave Resort, a small outdoor restaurant and cabins. The space was illuminated with infrared lighting, powered via outlets from the restaurant, and a single incandescent bulb was placed in the center on the ground to attract insects

[10] The entire data set includes 16 *H. alongensis* (5 females, 11 males) and 15 *H. grandis* (7 females, 8 males) that were included in either the obstacle (n = 9) or no obstacle (n = 22) condition.

into the space. Thin rope was strung across the top of the tent to allow for placement of hanging obstacles as well as tethered insects.

Bats were placed one at a time into the tent in one of two conditions: open (n = 6 bats; 3 males, 3 females), in which there were no obstacles placed, and cluttered (n = 6 bats; 5 males, 1 female), in which eight obstacles were placed throughout the space. Obstacles were constructed of thin rope with leaves taken from plants found at the study site attached to them. Individuals were allowed to fly freely for 15 minutes, with a single interruption at 10 minutes to provide additional sync indicators (described below). During this time, bats could explore the environment, forage on available insects, or rest on a mesh wall. After 15 minutes, bats were recaptured from the tent, offered water, and released at their original capture sites. Individuals were not repeated across conditions to maintain the novelty of the environment.

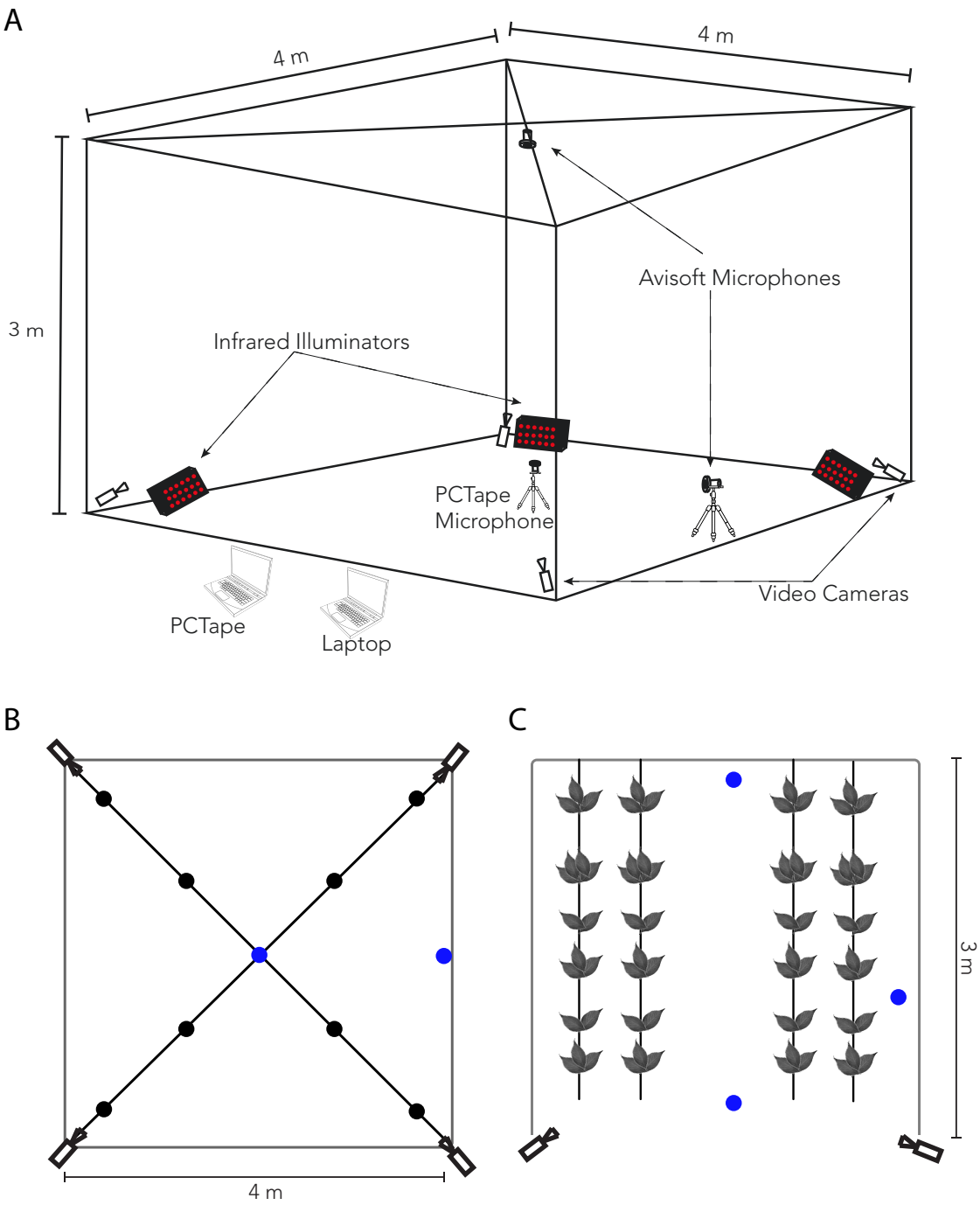

Figure 5.1. Renderings of the mesh tent located at the study site. Scaling is approximate. A) Depicts equipment required and its position with respect to the tent. Bats were released at ground level as the mesh had no doors/openings and this effectively contained the animals while also allowing them a place to perch. Obstacles (not shown) were hung from the lines strung diagonally across the top. The tent was supported with four bamboo poles. B) Top-down view of the enclosure show the placement of suspended obstacles (black circles) with respect to the cameras (each corner) and microphones (blue dots). C) Side view of the enclosure depicting obstacles made of vines and foliage with respect to cameras and microphones.

Video Recording, Processing & Analysis

Each trial was recorded with four high-speed, infrared sensitive camcorders (Zohulu, Shenzhen, China). Recordings captured in .mp4 format at either 30 frames s^{-1} (2560x1440 pixel resolution; first two nights) or 60 frames s^{-1} (1920x1080 pixel resolution; all subsequent nights) and included an audio track which was used for synchronization, but not acoustic analysis of bat calls. Files were transferred from SD cards to computers at the end of each night and backed up to external hard drives. Because of the harsh environmental conditions of the island forests, we used consumer-grade cameras, which lacked external synchronization methods. Recordings were started manually for each camera and synchronized with a sync indicator. This indicator was one of the experimenters kneeling in the center of the mesh tent, in view of all four cameras, and clapping their hands three times. This produced a brief auditory and visual signal that could be used to align all video and acoustic data streams. The sync indicator was provided at the beginning of each trial, prior to bat release, and after 10-minutes of the trial elapsed.

Manual synchronization of the four videos was completed in Adobe Premiere (Adobe Systems, San Jose, CA, USA) by editing the video to include only the sync indicators and the calibration segment or bat trials of interest. The audio tracks of each video were used to synchronize all frames and these new aligned videos were then individually exported to new .mp4 files for digitization.

From within each 15-minute recording, I extracted 1-8 second clips of the bats while performing each the three behaviors described in Table 5.1. Breaking the flight trajectory into these categories allows for a more straightforward comparison among

different individuals as each bat displayed varied patterns of behavior once released. Some were prone to attempting to escape and spent the majority of their time testing the mesh for exits while others flew almost non-stop and others barely flew at all after their initial release.

Table 5.1. Definitions of flight behavior categories.

Behavior	Definition
Release flight	The moment the bat flew from the experimenter's hand until the bat landed on one of the walls or three seconds had passed
Free-flight	Periods of continuous flight during which the bat had been in flight for at least 3 seconds prior
Alighting flight	From the moment the bat took flight after landing on one of the mesh walls

Three-dimensional reconstruction

Cameras were calibrated using the 'wand calibration' method described in Theriault *et al.* (2014) to allow for 3-D reconstruction of the flight trajectory of each bat. Calibration took place at the beginning of each trial night and each time cameras may have been disturbed during the course of the experiments by spinning a wand with two spherical, reflective points throughout the volume of the tent. Digitization of the wand points and calibration was conducted in MATLAB using the packages DLTdv5 and easyWand5 (Hedrick 2008; Theriault *et al.* 2014). Digitized bat trajectories were analyzed using custom MATLAB software for calculating kinematic parameters[11]. Missing frames were interpolated and data were smoothed using a quintic smoothing spline.

[11] An overview of this custom software can be found in the Appendix.

Kinematic Measurements

The smoothed 3-D bat trajectories were used to extract five kinematic parameters (Table 5.2). Calculation of these parameters was based on the methods described by (Combes *et al.* 2012). For each flight behavior category and obstacle condition, the mean and maxima of velocity, acceleration, and turn rate along with the average and minimum of turn radius were determined.

Table 5.2. Definitions of kinematic parameters.

Kinematic Parameter	Definition
Velocity (m s^{-1})	$v(t) = \frac{\Delta p}{\Delta t}$, where *p* is 3D position and *t* is time
Linear Acceleration (m s^{-2})	$a(t) = \frac{\Delta v}{\Delta t}$
Radial Acceleration (m s^{-2})	$a_{rad,i} = \frac{(v_{i+1} v_i)}{r_{curv,i}}$
Turn Rate (deg s^{-1})	$TR = \frac{\Delta \theta_{ITA}}{\Delta t}$, where θ_{ITA} is the idiothetic turning angle, or angular change in the direction of subsequent 3D velocity vectors
Turn radius or radius of curvature (m)	$r_{curv,i} = \frac{\lvert p_{i+1} - p_i \rvert}{[2\sin(\theta_i)]}$, where θ_i is the change in angle between velocity vectors v_i and v_{i+1}

Statistical Analysis

All statistical analyses were carried out in R v. 3.6.3 (R Core Team 2020). I performed two-way ANOVAs to determine the effect of sex on weight and forearm length. These are two commonly used metric for characterizing an individual's size. I tested the distributions of kinematic parameters for normality using Shapiro-Wilk tests and by examining descriptive plots. A reciprocal transformation was applied to linear acceleration, turn rate, and turn radius variables to achieve normality. I used a four-way

ANOVA, incorporating the obstacle condition, individual bat, bat sex, and flight behavior category for each of the five kinematic variables. Post-hoc pairwise comparisons on significant main effects was conducted using the estimated marginal means with *p*-values adjusted using the false discovery rate method (Benjamini and Hochberg 1995).

Because several parameters of flight performance will likely possess relationships with one another, I performed a principal factor analysis to examine which flight variables could potentially be explained by the existence of a common latent variable. To determine if this method is appropriate, I conducted Bartlett's test of sphericity (Bartlett 1951), which tests the overall significance of all the correlations within the correlation matrix. The test was significant ($\chi^2_{(10)} = 3774.44$, $p < 0.001$), indicating that it was appropriate to use factor analysis. I then used a combination of parallel analysis and visual inspection of a scree plot (showing the eigenvalues of principal factors compared to the factor number) to select the number of factors to retain for analysis (Glorfeld 1995). The loading matrix was rotated using the Varimax rotation to obtain orthogonal factors while retaining variables with loadings greater than 0.3 cutoff for inclusion (Hair *et al.* 2009).

Results & Discussion

I present a description of semi-captive flight behaviors in *H. a. alongensis*, a species with declining populations in Vietnam, during flight in either open or cluttered conditions within a mesh tent erected at the study site. 3D reconstructions of their flight trajectories (Fig. 5.2) were used to evaluate metrics of flight performance with respect to the presence of clutter and across individual animals.

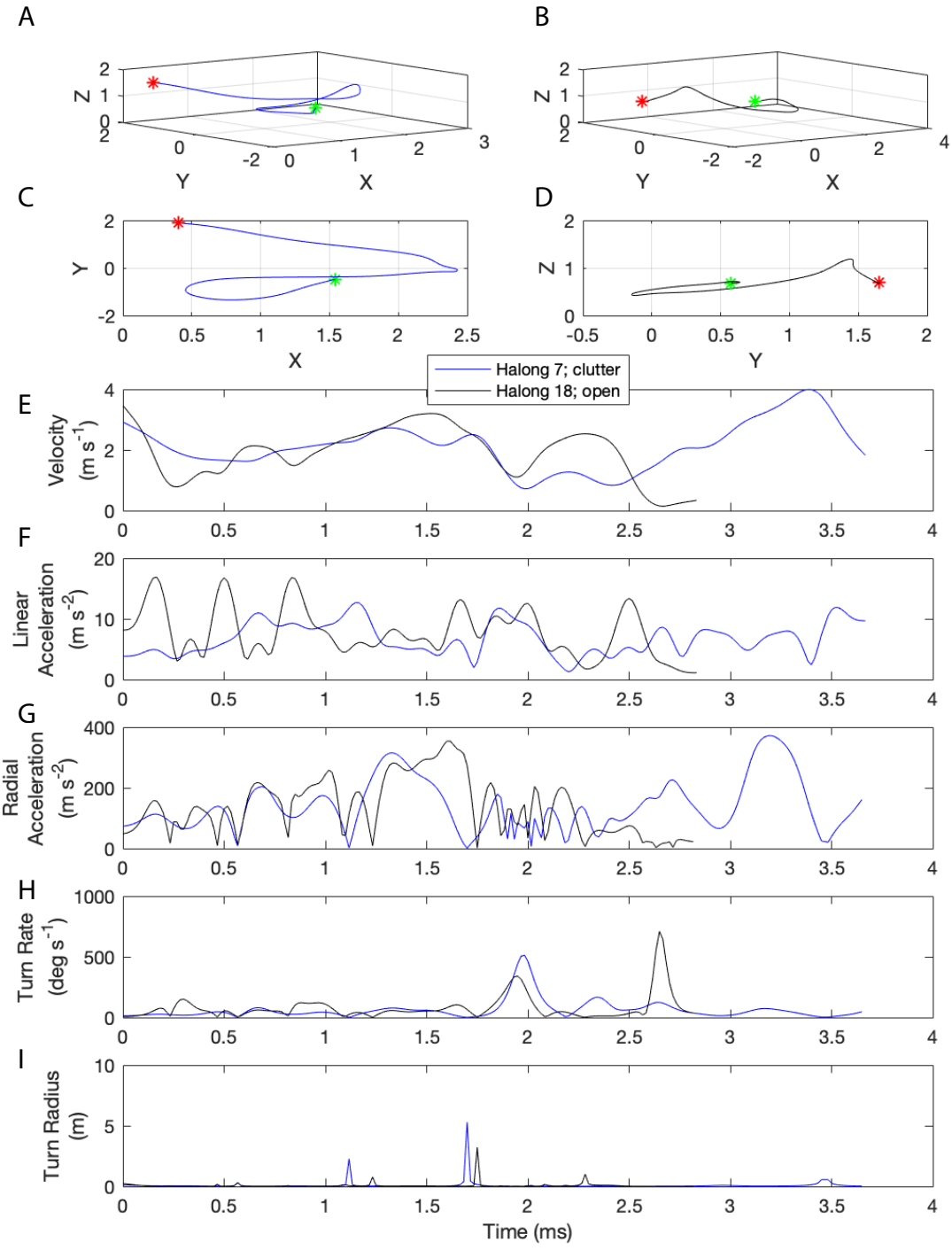

Figure 5.2. Example trajectories and their extracted kinematic parameters in open and cluttered conditions. These are two free flight trajectory segments from the bat Halong7 (blue lines) in the clutter condition with obstacles and Halong18 (black lines) in the open condition. Panels A and B are 3D views while panel C shows a top-down view and panel D shows a lateral view. In panels A-D, green asterisks represent the start of the trajectory while red asterisks represent the end. Panels E-I describe the fluctuations in each kinematic parameter over the duration of each trajectory.

Effects of Obstacles

Clutter in the tent was created by hanging several obstacles made of natural materials within the tent, spanning ceiling to floor, to compel bats to avoid collision as they flew through the space. The obstacles had a significant impact on multiple kinematic parameters (Fig. 5.3) and average and maximum values for each parameter are reported in Table 5.3. The presence of obstacles resulted in a small but significant decrease in average flight velocities compared to the open condition ($F_{(1,21)} = 4.73$, $p = 0.041$) along with higher radial accelerations ($F_{(1,21)} = 5.95$, $p = 0.024$). Bats also exhibited increased turn rates ($F_{(1,21)} = 50.78$, $p = 0.0088$) and significantly smaller turn radii ($F_{(1,21)} = 32.00$, $p < 0.001$) in cluttered conditions.

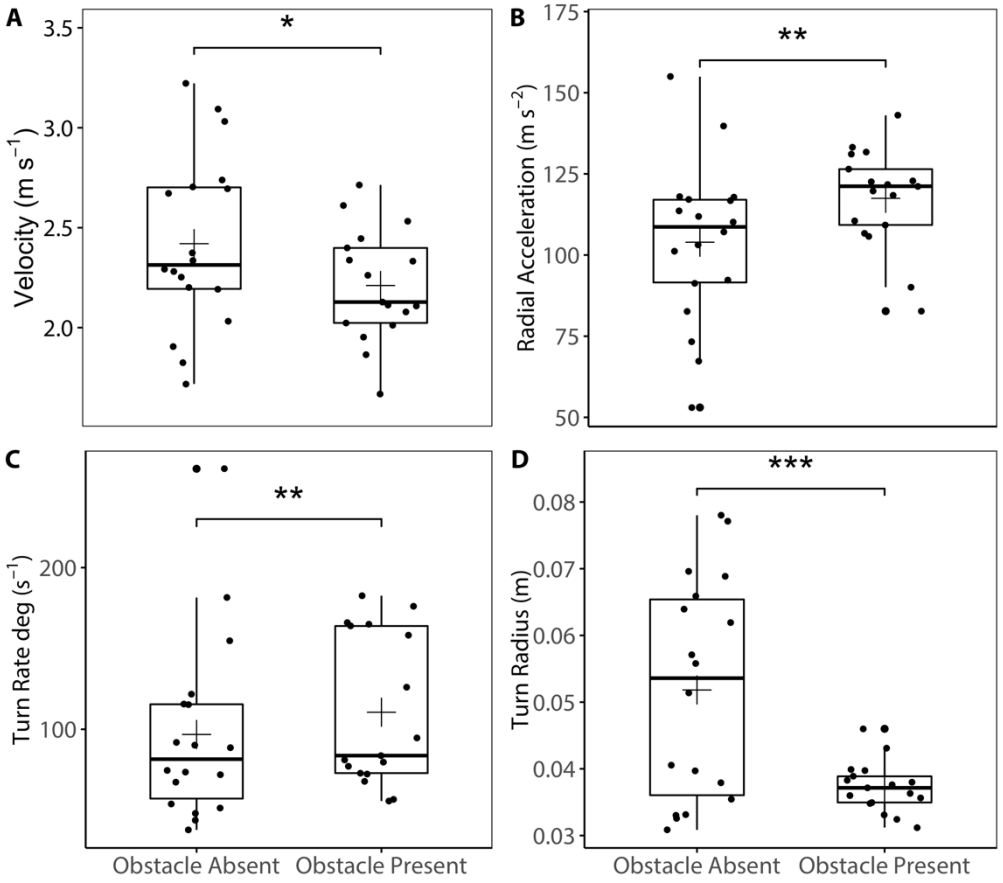

Figure 5.3. The effect of obstacles on kinematic parameters. A) Velocity decreases when obstacles are present. B) Radial acceleration increases in the presence of obstacles. C) Turn rate is less in the open condition. D) Turn radii are greater in the absence of obstacles. Linear acceleration did not change significantly across conditions and is not depicted here. Black dots represent the average value for each trial that makes up the dataset. The plus symbol represents the mean values. Significance levels are as follows: $p < 0.001$ '***'; $0.001 < p < 0.01$ '**'; $0.01 < p < 0.05$ '*'; $p > 0.05$ 'NS'

Factor Analysis of Kinematic Parameters

Kinematic parameters are often highly correlated with each other as they are all subject to the same biomechanical constraints of animal anatomy and physiology. Looking at individual variables can be informative to understand the specific changes occurring throughout the flight trajectory, but reducing these variables into broader, underlying descriptions of motion can increase interpretability.

I carried out a principal factor analysis with a Varimax (orthogonal) rotation of four of the five kinematic variables extracted from the trajectory data whose loadings were above the designated cutoff criterion. From the factor analysis, two factors are retained which captured the variance of four variables – velocity, radial acceleration, turn radius, and turn rate (Table 5.4). Factor 1 and Factor 2 had sum of squared loadings values of 1.48 and 1.41, respectively and explained 37% and 35% of the total variance in the data, respectively. No matter the rotation type, a simple structure could not be obtained resulting in velocity being cross-loaded onto both factors.

These factors encompass two of the primary characteristics traditionally used to characterize sustained flight in animals: maneuverability and agility. Agility is generally regarded as a measure of the ease and rapidity with which a flight path can be altered and, in this case, is described by velocity and radial acceleration. Maneuverability refers to the space required to alter a flight path and incorporates measures of turn rate and the turn radius. Velocity also cross-loads onto the maneuverability factor as overall velocity likely constrains these parameters (Norberg and Rayner 1987). Thus, general agility increases in the presence of environmental clutter with slower overall velocities but increases in the forward velocity during a turn leading to faster alterations in flight paths. Overall flight maneuverability also increased with bats performing tighter turns and increasing turn rates.

Table 5.3. Factor loadings of kinematic variables.

Parameter	Factor 1 Loading	Factor 2 Loading
Velocity ($m\ s^{-1}$)	0.79	0.59
Radial Acceleration ($m\ s^{-2}$)	0.92	–
Turn Rate ($deg\ s^{-1}$)	–	0.37
Turn Radius (m)	–	0.92

Effect of Sex and Individual

Most mammals exhibit some sort of sexual dimorphism, including bats for which there have been documented differences in physical characteristics (Storz *et al.* 2001 Jan 1; Ulian and Rossi 2017; Wu *et al.* 2018) and echolocation (Kazial and Masters 2004; Thomas *et al.* 2004; Grilliot *et al.* 2009; Fu *et al.* 2015). Body size in particular has been documented to be either female-biased or absent (Myers 1978; Stevens *et al.* 2013; Williams 2020). We also know that these same features can vary within individuals (Masters *et al.* 1995; Kazial *et al.* 2001b; Voigt-Heucke *et al.* 2010). Since flight performance is dictated by body size and morphological characteristics, which is in turn influenced by ecological requirements and sensitive to such changes[12], we would expect that sexual dimorphism and individual variation in physical traits would result in corresponding differences in flight abilities.

For this preliminary set of data, we only measured weight and forearm length in the field. Forearm lengths for *H. a. alongensis* typically range from 68 mm to 76 mm while weights vary between 22 g and 35 g. We note sex differences in forearm length ($F_{(1,26)}$ = 29.53, $p < 0.001$; Fig. 5.4a), with males (M = 70.1 mm, SD = 1.27 mm) having longer forearms than females (M = 69.06 mm, SD = 2.01 mm). We did not note any differences in weight between sexes ($F_{(1,26)} = 0.031$, $p = 0.35$). Analysis of flight behaviors show that females tended to fly faster than males ($F_{(1,21)} = 4.90$, $p = 0.038$). Males, however, had

[12] Females are subject to a greater range of changes in weights that they must carry due to pregnancy and the transportation of young. Although no females appeared to be pregnant or lactating and none were carrying young. We conducted experiments outside of the time they typically give birth (usually prior to May or June) and during a time when they were becoming reproductively active (July to September).

overall greater accelerations ($F_{(1,21)} = 36.63$, $p < 0.001$) and faster turn rates than females ($F_{(1,21)} = 50.78$, $p = 0.0088$). Significant individual differences in the bats' performance are outlined in figure 5.4b-d.

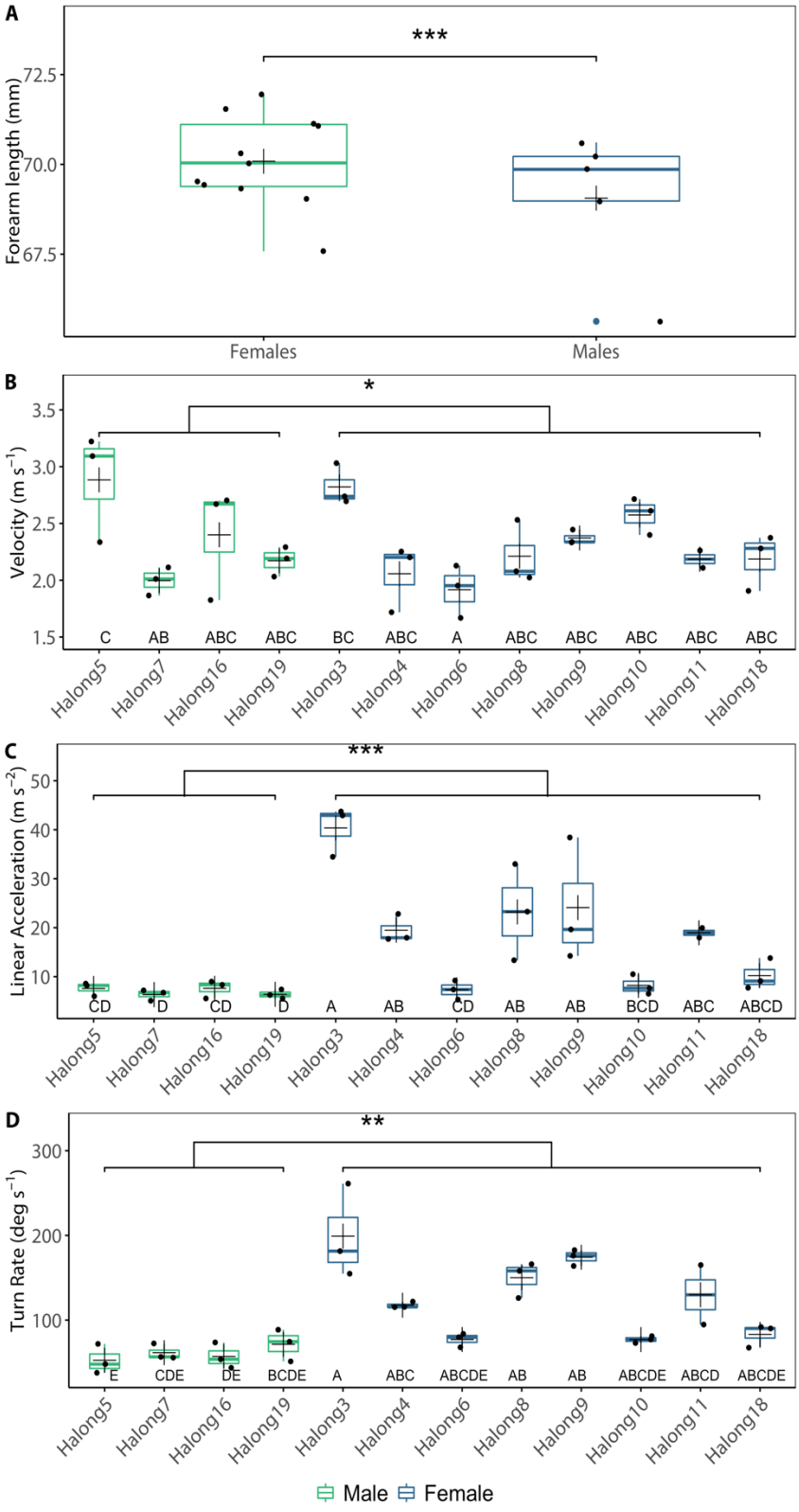

Figure 5.4. Individual and/or sex differences in forearm length and kinematics. A) Females have longer forearms. Velocity (B), linear acceleration (C) and turn rate (D) all vary significantly between sexes and show individual differences. Black dots represent the average value for individual bats. The plus symbol represents the mean values. Significance between sex is indicated by bars at the top of the plot and significance between individuals is represented by the compact letter display at the bottom of the plot. Significance levels are as follows: $p < 0.001$ '***'; $0.001 < p < 0.01$ '**'; $0.01 < p < 0.05$ '*'; $p > 0.05$ 'NS'

High resolution photographs of the wings of live bats are expected to be taken for the expanded version of this study that incorporates echolocation and detailed morphological characteristics. Low resolution photographs of individuals were taken prior to inclusion in the experiment but we do not analyze these here, primarily due to the imbalance between sexes in the conditions and the experiment as a whole. This combined with the absence of additional morphological data make the differences reported due to sex, though potentially interesting, likely to result in unreliable measures. Ideally, bats would be flown multiple times in both the open and cluttered conditions, to give a better indicator of individual variability. This combined with a larger sample size with more equal numbers of each sex would present a more robust analysis.

Broader Impact of the Study

Few detailed studies quantifying the adaptability of flight behaviors have been conducted to date partially due to the difficulty in acquiring such measurements. The goal of this study was to take an ecological approach to studying aerial locomotion in a reproducible manner to update and augment the meta-analysis published by Norberg and Rayner (1987), which contains several shortcomings that have the potential to impact reported metrics. For example, no distinction in sex or age classes of specimens used is documented.

Most notably, morphological characteristics are drawn from unpublished data or personal communication with no way to verify consistency in measurement taking.

Approximately 22% of physical measurements were obtained from preserved specimens, which are known to create significant deviations in body mass and wing measurements compared to live specimens (Bininda-Emonds and Russell 1993).

Another drawback is that speed is the only kinematic parameter measured and it is done so inconsistently, making it difficult to create meaningful comparisons from which to draw conclusions. For example, methods of measurement ranged from using car or boat speedometers (Goodwin 1928; Bloedel 1955; Twente 1955) to stopwatches (Hayward and Davis 1964) to stroboscopic photography (Habersetzer 1981), the predecessor of high-speed video recordings. Some papers simply list "direct observation" as the method for determining speed (Belwood and Fullard 1984). Consistent flight contexts could also not be established; some bats were flown indoors in controlled conditions (Hayward and Davis 1964) while some were measured in homing (displacement) experiments (Cockrum 1956; Mueller and Emlen 1957). We would expect that bats performing different tasks like commuting to different sites, foraging, or avoiding predation would show high variability in flight speed and other parameters. Environmental and goal/task context will determine average vs. peak performance metrics.

While our methods may not be ideal, they have the ability to provide consistent data output that is comparable across studies. Ideally, we would collect longer, uninterrupted flight segments in a larger area than that provided by the tent. Since our segments were so limited (frequently punctuated by landings or obscured due to insufficient camera coverage or recording failures), we can only make comparisons relative to the flight behavior categories outlined in the methods. However, creating more

strictly defined categories as the bat flies in different environments will create equivalent contexts to document task- and habitat-specific behaviors, including those that might prompt peak performance in kinematic parameters such as pursuit of highly evasive prey items (Corcoran and Conner 2016).

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Appendix

Software for the calculation of kinematic parameters in 3D animal trajectories

Chapter Notes

I first began writing the scripts that make up this module as a Master's student at Wake Forest University. In addition to exploring sonar jamming, I was studying how bats alter their flight trajectories when pursuing different types of insects. I wanted to use 3D reconstructions to quantify the wide variety of insect escape tactics that include erratic turns, dead drops, and last minute maneuvers and how different species of bat coped with this by using simple pursuit of predictive flight strategies. I largely failed in this endeavor: WFU had a very small flight room and no captive colony so most of my work was limited in season and geography to specific field sites with access to power for all the equipment. But I could still use the code to analyze flight data for other experiments and it has become an indispensable part of the analyses in this dissertation.

Summary

Quantifying the movements of an animal moving in three dimensions is challenging. Despite numerous programs, that allow for the collection of these datasets, software for this application is scarce, with a few highly priced commercial options. Even more challenging

is the integration of the computation of kinematic parameters with the determination of an animals' acoustic emissions in synchrony with the motion trajectory. In the absence of affordable commercial software and fully developed open source software, here we created two MATLAB modules that would integrate positional and acoustic data generated by a synchronized data collection system. This software is generalizable to any data set composed of comma separated values of Cartesian data points in the form of *x*, *y*, and *z* coordinates of position data along with the known temporal information (i.e. frame or sampling rate). It is also designed to be used by those who have only a basic understanding of programming in an effort to make animal motion analysis feasible for across numerous disciplines. The code will be available post-dissertation submission on GitHub at github.com/tekjones/.

Background

Much of what we know about natural guidance systems (Ghose 2006; Tammero and Dickinson), pursuit (Land 1993; Mizutani *et al.* 2003; Ghose *et al.* 2006), and performance (Fry *et al.* 2003; Tian *et al.* 2006; Wang and Russell 2007; Shelton *et al.* 2014) are based on experiments in which the animal was tracked during flight and measurements taken from its reconstructed trajectories. These motion paths are studied across diverse field including population ecology, neuroethology, and conservation biology and are used to answer questions about navigation, foraging, dispersal, and inter- and intraspecific interactions (Pyke *et al.* 1977; Turchin 1996; Mouillot and Viale 2001; Gulesserian *et al.* 2011; Shamble *et al.* 2017).

Animals in flight, such as insects, birds, or bats, and aquatic organisms all regularly create motion paths outside of the simple *x* and *y* coordinate system. This extra dimension of motion is often captured using a system of two or more calibrated cameras (Hedrick 2008), yielding datasets in the form of video files. The ability to analyze freely behaving animals through videos is a desirable technique because it is minimally disturbing to the subjects, often only involving the placement of reflective markers. However, with the rise of advanced image and video analysis, such as those employed by DeepLabCut (Nath *et al.* 2019), even this requirement is disappearing, making partial and full automation of 3D coordinate extraction from video sets possible.

Extracting 3D coordinates can be done in numerous software packages and the simple measure of position can be further extrapolated into more advanced kinematic measurements such as velocity, acceleration, and various angles. These measurements

provide a quantitative characterization of animal motion that makes the process of describing and comparing trajectories more reliable, less biased, and reproducible. Unfortunately, calculating these measurements is not always straightforward and currently, few software packages are available. Elementary processing for single trajectories such as the distance between subsequent point pairs and speed along with simple visualizations, can be achieved with a software package called Superplot3d (Whitehorn *et al*. 2013). Proprietary software can provide some of these extended measures, but many are specifically focused on the analysis of human motions, or are restricted to gait or joint flexion analysis. In addition to being costly, these options are rarely customizable to the unique applications of individual research paradigms.

This software package differs from previous applications in four critical ways. Firstly, the software allows for the simultaneous analysis of two trajectories in relation to each other and to a third static data point, such as a microphone, support up to an n x 9 matrix of values. Secondly, it easily provides the calculations of kinematic parameters that are otherwise difficult to derive from position data. Thirdly, it provides a basic method for automatically interpolating missing data and smoothing noisy data that arises through errors in the digitization process. Lastly, rather than processing individual files, users have the option to evaluate large collections of files, provided they are organized appropriately.

The current drawbacks and caveats of this program are that users must have a working MATLAB license in order to ensure their starting data are properly formatted and to be able to read in data and export the processed files. This also means that the user must have basic familiarity with MATLAB variables such as structs, in order to make use

of the functions. Unlike professionally developed software, this does not allow for the input of a large number of points, like one might associate with a marker-based "skeleton" of an object, though future iterations have the potential to incorporate this.

The next step for this project is to turn it into a standalone executable using the MATLAB compiler toolkit, so that users do not need a costly MATLAB license to use it. Alternatively, conversion into a more widely used non-engineering-oriented programming language such as Python or R would maximize the availability of the code. Ideally, the implementation of a graphical user interface for simpler execution and the easier creation of basic visualizations for preliminary data inspection will be part of future development.

IMPLEMENTATION OF THE TRAQR MODULE

Although animal trajectories are continuous over time, data are typically obtained and analyzed as discretely sampled locations and time points. The Traqr module is designed to handle position data that was collected at constant time intervals. Data that were sampled at irregular steps will need additional pre-processing by the user to yield accurate information from this software.

Users currently interact with a front-end MATLAB script (Fig. 1) that allows for single file, or whole directory processing and allowing for easy manipulation of the required input variables. The module requires four user inputs: the .CSV file, the frame rate at which the video was recorded (fps), target type (static or dynamic), and an indication of whether they have included the positional data of a microphone (software assumes that microphone is stationary).

The first point of interest (POI_1) is typically the trajectory of the animal of primary interest in the study. "Target" is used to refer to the second point of interest (POI_2) and may be a conspecific, a prey item being pursued, or a stationary object like a landing platform or obstacle.

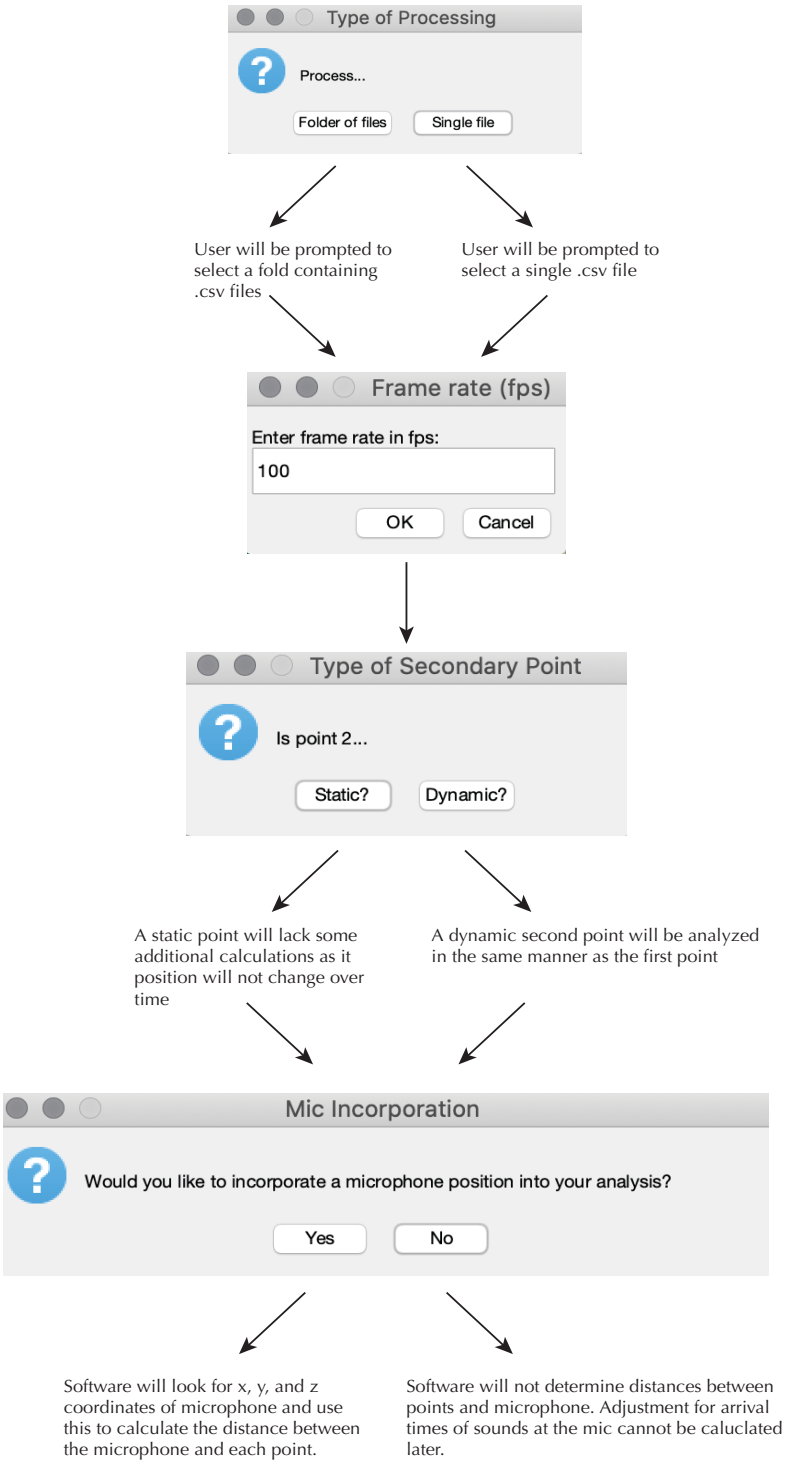

Figure A.1. Traqr frontend prompts. First series of prompts a user interacts with upon running the Traqr_Frontend software. Software is initialized with the command line prompt:

```
>> Traqr_Frontend
```

Each trajectory is represented internally as a series of four-dimensional coordinates (*x*, *y*, *z*, time). If two trajectories are being analyzed simultaneously for comparison and they are of unequal lengths, the inputted matrix is abbreviated to the length of the shortest trajectory. Missing datapoints are then linearly interpolated to generate estimated positional data in all three dimensions[13]. The trajectory of each point of interest is usually embedded in some level of noise and there are several methods that can be used to attenuate this noise when calculating additional parameters, like velocity and acceleration. Low-pass Butterworth filters are often used in gait analyses, such as human walking trajectories (Schreven *et al.* 2015), and curve fitting using splines is often implemented in studies of flight. This software makes use of the latter, in which a smooth curve is fitted over a small subset of adjacent data points before moving onto the next grouping of data points, repeating the fitting process until the end of the data structure is reached. This provides localized smoothing that is effective in removing data errors (Winter 2009) and quintic spline algorithms in particular have been shown to provide good estimates of motion parameters (Walker 1998).

From this data, the kinematic parameters for each time point in each trajectory are calculated using methods described by Combes *et al.* (2012). Distance and angle measurements at each time point are also generated between the two trajectories and each trajectory and the microphone if present. Output variables are currently stored as six

[13] Trajectories with large gaps of missing data should be treated with care. While linear interpolation is useful, it can result in unreliable measurements and should be reserved for small time gaps, depending on the frame rate at which data were collected.

struct variables in .MAT file formats: Angles, POI_1, POI_2, PM, PT, and Time. Table 1 defines and describes the contents of each struct variable.

Table 1. Output of Traqr module.

Parameter	Definition
XYZ	The smoothed XYZ coordinates generated by evaluating the spline function.
Spline	B-form of the smoothest function.
Velocity (m s^{-1})	Calculated for POI_1 and POI_2. Returns 3D velocity and decomposed velocity in the horizontal and vertical planes.
Linear Acceleration (m s^{-2})	Calculated for POI_1 and POI_2. Returns 3D linear acceleration and decomposed linear acceleration in the horizontal and vertical planes.
Radial Acceleration (m s^{-2})	Calculated for POI_1 and POI_2. Returns the magnitude and accounts for forward velocity in maneuverability.
Frenet Trihedron	Calculates the unit tangent, unit normal, and unit binormal.
Distance	Calculates the distance between POI_1 and POI_2 and the distance between POI_1 and the microphone.
Radius of curvature (m)	A measure of how widely the animal turns.
Time	Produces the absolute frame number of each 3D position, the absolute time in seconds, and the relative time to the shortest distance between POI_1 and POI_2.
Angles (°)	For POI_1 and POI_2 , calculates the angles, decomposed angles in the horizontal and vertical planes, and their first time derivatives.
Alpha	Angle between the reference vector (x-axis) and the vector connecting POI_1 and POI_2.
Beta	Angle between the vector connecting POI_1 and POI_2 and the tangent of POI_2.
Gamma	Angle between the reference vector and the tangent of POI_2.
Theta	Angle between the reference vector and the tangent of POI_1.
Phi	Angle between the vector connecting POI_1 and POI_2 and the tangent of POI_1. Also generates optimum angle based on CB/CATD strategy.

IMPLEMENTATION OF THE ECHOLOCATOR MODULE

The sounds an animal generates while performing some task, whether it is foraging for food, navigating the environment, or communicating with conspecifics combined with knowledge of its movements can provide unparalleled insight into a variety of processes like orientation, decision-making, and other aspects of daily life. For animals that rely heavily on acoustic forms of auto-communication, like bats or dolphins, alterations in the acoustic parameters are indicators of activity.

The EchoLocator module (Fig. 2) is designed to incorporate the temporal information associated with the animal creating the associated motion path and determine where along the trajectory distinct calls are occurring. This allows for the simultaneous analysis of spectrotemporal parameters and the calculation of distance-related measures. Users need to input a file containing 1) a list of times for the beginning, or onset of each call, 2) the end, or offset, of the call, and 3) the sampling rate at which data were collected. The bat_vocal_analysis.m code written by Melville J. Wohlgemuth generates these features for frequency-modulated bat calls as after semi-manual adjustment of a call-detection algorithm and saves them to a .MAT file. This is what was used in the creation of the scripts, but any acoustic analysis software could be used (e.g. Avisoft SASLab Pro, Audacity, Praat, Adobe Audition). Users also need to have available the trajectory information that was generated from the Traqr module.

The output of the EchoLocator module is described in Table 2. Because animals are often moving in relation to a stationary microphone doing the audio recording, the module uses the speed of sound (343 m/s) and the distance of the animal to the

microphone at each position interval, to compensate the timing of the sound, making measures of duration and inter-call intervals more accurate. Each call will also be assigned to a distance bin relative to the "target" location at that time point.

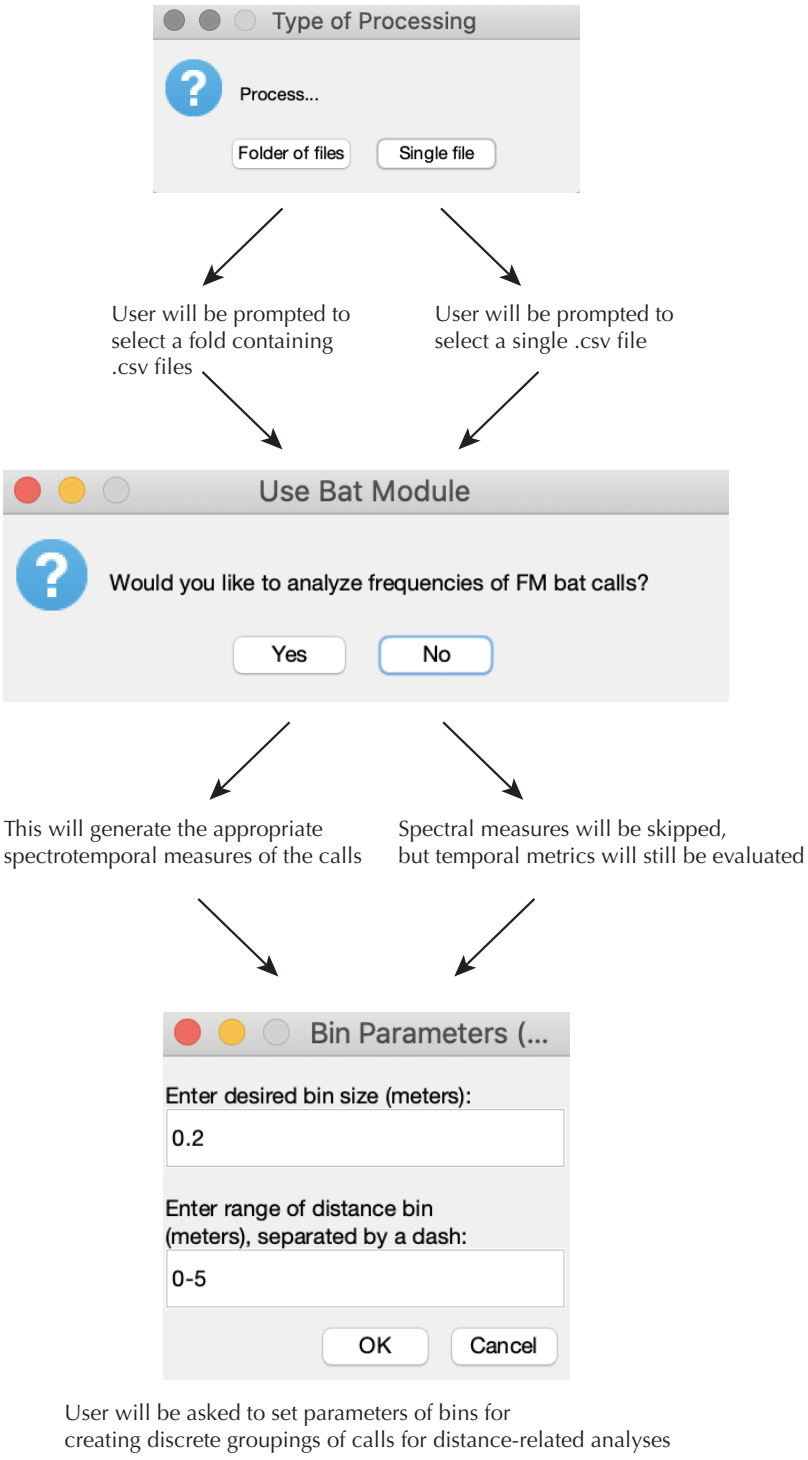

Figure A.2. EchoLocator frontend prompts. First series of prompts a user interacts with upon running the EchoLocator_Frontend software. Software is initialized with the command line prompt:

```
>> EchoLocator_Frontend
```

Table 2. Output of EchoLocator module. These metrics are currently specific to ultrasonic emissions of bat calls, but minimal adjustment in the code could expand this to include the vocalizations of any moving organism.

	Acoustic Parameter	Definition
	XYZ	The *x, y,* and *z* coordinates along the animal trajectory at which each call occurs.
Spectral	Peak frequency (kHz)	Frequency (kHz) with the most energy in a call.
	Start frequency (kHz)	Frequency at beginning of each call - 20dB below peak frequency.
	End frequency (kHz)	Frequency at end of each call -20dB below peak frequency.
	Bandwidth (kHz)	Range of frequencies in a sonar emission.
Temporal	Onsets and offsets of calls	Adjusted to compensate for sound source motion using the distances of POI_1 to the microphone and the speed of sound. Calls that occur outside the of the calibrated 3D volume will be skipped.
	Call interval (ms)	Time between successive call onsets.
	Call duration (ms)	Duration of individual sonar emissions.
	Sweep rate (kHz/ms)	Quotient of bandwidth ÷ call duration; describes the slope of a frequency modulated call.
	Sonar Sound Groups	Clusters of echolocation calls with similar call intervals embedded in a sequence of calls with longer call intervals.

Te K. Jones

Curriculum vitae

PROFESSIONAL SUMMARY

- Biopsychologist with experience in the collection, management, and quantitative and qualitative analysis of large datasets involving behavior, signal processing, 3D reconstructions, and computer vision using programming languages such as MATLAB
- Independently pursued the study of data science techniques utilizing R and Python with an emphasis on object detection and supervised machine learning
- Demonstrated excellent oral and written communication skills as evidenced by 5+ years of teaching experience, 12 scientific publications and presentations, and collaborations with researchers and non-researchers

EDUCATION

Johns Hopkins University ■ Baltimore, MD 2018 - Present
Ph.D. | Psychological and Brain Sciences

Johns Hopkins University ■ Baltimore, MD 2018 - 2019
M.A. | Psychological and Brain Sciences

Wake Forest University ■ Winston-Salem, NC 2015 - 2018
M.S. | Biology

Salem College ■ Winston-Salem, NC 2011 - 2014
B.A. | Biology

RESEARCH EXPERIENCE

Ph.D. Researcher ■ Johns Hopkins University ■ Baltimore, MD 2018 - Present

- Designed experiments to study sensory information processing and adaptive motor control in echolocating bats to understand the perceptual underpinnings of natural behaviors
- Implemented custom synchronized high-speed camera and microphone arrays for the collection of audio and kinematic data in laboratory and field settings, streamlining the data collection process
- Consulted on research initiatives focused on transferring information on natural flight in insects to military technology with scientists at the Eglin Air Force Base
- Managed the project progress of 9 junior researchers in laboratory and field settings ensuring fulfillment of academic credit requirements
- Established collaborations with researchers in the department of biomedical engineering to study the vestibular system and perception of self-motion in bats
- Authored technical guides for the preservation of laboratory and field techniques
- Independently created custom data analysis pipelines and code modules to extract and process 3D kinematic measurements from flight position coordinates, reducing data processing times from several weeks to a single day

M.S. Researcher ■ Wake Forest University ■ Winston-Salem, NC 2015 - 2018

- Independently conducted experiments to document the reproductive behaviors of sympatric tiger beetle communities to understand the importance of color patterns in mate choice
- Spearheaded two data collection expeditions in the field to study predator-prey interactions between bats and insects to quantify the acoustic changes associated with sonar jamming and other forms of acoustic interference
- Led 3 workshops on scientific writing and communication geared towards technical and non-technical audiences
- Designed and led 2 workshops on applying to the NSF Graduate Research Fellowship program for senior undergraduates and first year graduate students

Research Resident ■ UNC Center for Design Innovation ■ Winston-Salem, NC 2013 - 2015

- Collaboratively researched the relationships between flight morphology and prey capture performance in bats using kinematic technologies and documented group behavior in Brazilian free-tailed bats during cave emergence and return in the field
- Produced lectures around scientific data literacy including how to create impactful visualizations and ethical considerations
- Implemented LiDAR laser scanning technology to build 3D point-cloud reconstructions of cave morphology of bat-inhabited caves in the southwestern United States.
- Provided guidance on a collaborative project on motion analysis of people with Parkinson's disease before and after improvisational dance
- Assisted with the deployment of the pilot program of Interpreters and Scientists Working on our Parks (iSWOOP), an educational program for park rangers at National Parks designed to facilitate relationships with working scientists and enhance the informal learning experience of park visitors

PUBLICATIONS

- **Jones, T.K.**, Moss, C.F. (2020). Behavioral enhancement by visual cues during obstacle avoidance in echolocating bats. (in preparation).
- **Jones, T.K.***, Diebold, C.A.*, Moss, C.F. (2020). Quantification of comparative flight and echolocation in *Hipposiderids* in cluttered environments. (in preparation).
- **Jones, T.K.**, Allen, K.A., Moss, C.F. (2020). Perspectives on signal jamming and the jamming avoidance response. (in preparation).
- **Jones, T.K.**, Conner, W.E. (2019). The jamming avoidance response in echolocating bats. *Journal of Communicative & Integrative Biology*.
- **Jones, T.K.**, Wohlgemuth, M.J., Conner, W.E. (2018). Active acoustic interference elicits echolocation changes in heterospecific bats. *Journal of Experimental Biology*.
- **Jones, T.K.** and Conner, W.E. (2018). Pre-mating reproductive isolation in tiger beetles (Cicindelidae): an Examination of the Role of Visual and Morphological Feedback. *Journal of Insect Behavior*.

SCHOLARLY PRESENTATIONS

- International Field Course in Animal Communication ■ Cát Bà, Hải Phòng, Vietnam 2019
- International Congress of Neuroethology ■ Brisbane, QLD, Australia 2018
- Nature-Inspired Flight Technologies + Ideas ■ Seattle, WA, USA 2018
- Society for Integrative and Comparative Biology ■ San Francisco, CA, USA 2018
- Society for Integrative and Comparative Biology ■ West Palm Beach, FL, USA 2015
- Salem College Women in Science and Mathematics ■ Winston-Salem, NC , USA 2013

TEACHING EXPERIENCE

Teaching Assistant ■ Johns Hopkins University ■ Baltimore, MD 2018 – 2019

- Advanced Statistical Methods ■ 1 semester
  - Equipped 10 early-stage graduate researchers with the analytical tools required to select, apply, and interpret the results of statistical techniques with emphasis on application in psychological research
  - Introduced R programming language for data management and analysis
  - Created custom content in SWIRL for learning the basics of R programming in an interactive manner
- Advanced Research Design and Analysis ■ 1 semester
  - Facilitated the understanding of complex research designs and analytical techniques
  - Provided individual tutoring on independent projects designed to apply data management and statistical frameworks for independent projects

Laboratory Instructor ■ Wake Forest University ■ Winston-Salem, NC 2015 - 2017

- Comparative Physiology Lab ■ 4 semesters
  - Taught basic skills associated with the design, execution, and reporting of experiments to 120+ undergraduate-level researchers
  - Created lectures to guide students through the logic of identifying relationships between form and function

Guest Lecturer ■ North Forsyth Middle School ■ Winston-Salem, NC 2016

- Immunology ■ 1 class
  - Designed and implemented interactive teaching games for communicating basic knowledge about human DNA and its importance to immune systems to 25 middle school students

Teaching Assistant ■ Winston Salem State University ■ Winston-Salem, NC 2014 - 2015

- Introduction to Biology ■ 1 semester
  - Tutored 100+ students as they learned major concepts that are the foundation of modern biology and medicine
  - Evaluated student performance on quizzes and exams
- Principles of Biology ■ 1 semester
  - Created lectures on ecological principles and guided students through case studies designed to illustrate these principles

GRANTS ■ AWARDS ■ FELLOWSHIPS

- Insight Data Science Fellowship 2020
  - A training fellowship designed to give experience building data science and artificial intelligence solutions to ~5% of applicants with emphasis on data curation, analysis, documentation, and presentation
- Off-campus Research Expansion Award 2019
  - An award of $3,000 to supplement equipment and travel costs for research being conducted in collaboration with researchers outside the university
- Heiligenberg Student Travel Award 2018

    - $750 to cover travel and lodging to attend the International Congress of Neuroethology meeting awarded to ~5 students each meeting
- Charlotte C. Magnum Student Support Program Award 2018
    - Provides lodging to attend the Society for Integrative and Comparative Biology Meeting
- National Science Foundation Graduate Research Fellowship 2017
    - A graduate training fellowship awarded to ~12% of applicants that covers university tuition and provides a stipend of $102,000 over 3 years
- Elton C. Cocke Travel Award 2017
    - An award of $500 that reimburses the expenses of attending conferences
- Southwestern Research Station Student Support Fund Grant 2017
    - A grant to subsidize the cost of lodging at the SWRS field research site
- Vecellio Grant for Graduate Research 2016
    - A $1,000 grant for small research equipment purchases

SCIENTIFIC SOCIETIES

- Society for Integrative and Comparative Biology
- International Society for Neuroethology
- Acoustical Society of America

MENTORING & VOLUNTEERING

- Volunteer ■ Judge ■ *Science Olympiad* ■ 2017
- Mentor ■ Sylvia Baila ■ *Effects of playback timing on bat behavior* ■ 2016
- Mentor ■ Christina Benedict ■ *3D space calibration: wand vs. drone* ■ 2015 - 2016
- Mentor ■ Julianne DeMarco ■ *Tiger beetle mandible morphology* ■ 2017
- Mentor ■ Maria Pedraza ■ *3D reconstructions of bat flight trajectories* ■ 2018
- Mentor ■ Kevin Duffy ■ *3D reconstructions of bat obstacle avoidance* ■ 2019 - 2020
- Mentor ■ Paige Upright ■ *Temporal acuity of echolocating bats* ■ 2019 - 2020
- Mentor ■ Jessica Montoya ■ *Feature salience in echolocating bats* ■ 2019 - 2020
- Mentor ■ Jason Cheung ■ *Quantification of flight behaviors* ■ 2020
- Mentor ■ Zhuochen Yuan ■ *Quantification of flight behaviors* ■ 2020